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B. T. GALLOWAY, *Chief of Bureau.*

INVESTIGATIONS OF THE POTATO FUNGUS PHYTOPHTHORA INFESTANS.

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[In cooperation with the Vermont Agricultural Experiment Station.]

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COTTON AND TRUCK DISEASE AND SUGAR-PLANT INVESTIGATIONS.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., January 20, 1912.

Sir: I have the honor to transmit herewith and to recommend for publication as Bulletin No. 245 of the series of this Bureau the accompanying technical paper by Dr. L. R. Jones, Mr. N. J. Giddings, and Prof. B. F. Lutman, entitled "Investigations of the Potato Fungus Phytophthora Infestans."

This potato fungus causes the most destructive disease known to this important crop and has thus far baffled attempts at its complete control, owing probably in part to ignorance of its entire life history. This paper embodies not only a consideration of the main facts in the development of the disease and of the methods for its control by spraying and other standard practices, but also contributes some important new facts as to the development of resting spores by the fungus. Attention is further directed to the possibilities of control through the use of disease-resistant varieties, and a laboratory method is here developed for promptly testing the relative disease resistance of potato tubers.

The work has been done by Dr. Jones as a collaborator of the Bureau of Plant Industry, in cooperation with the Vermont Agricultural Experiment Station, with the assistance of Mr. Giddings and Prof. Lutman and of the workers mentioned in the footnote on the first page of the text. The need of such an investigation had long been felt and it was undertaken in this manner because of the long experience of Dr. Jones with this problem and because the disease is of much more frequent occurrence in Vermont than in the vicinity of Washington. The bulletin forms part of a series of studies on potato diseases and disease resistance in progress in this Bureau under the general direction of Mr. W. A. Orton.

The illustrations submitted are essential to an understanding of the text.

Respectfully,

B. T. Galloway,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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B. P. I.—719.

INVESTIGATIONS OF THE POTATO FUNGUS PHYTOPHTHORA INFESTANS.[1]

INTRODUCTION.

These studies upon the potato fungus *Phytophthora infestans* have been the outcome of apparent need coupled with evident opportunity. For both economic and scientific reasons there is need of a fuller understanding of the life history of this fungus and its relations to the host plant, while the frequency of its occurrence in Vermont has offered favorable opportunity for carrying on such investigations. No other of the staple farm or garden crops of this country is subject to such extreme variations in production as the potato, because of disease of one or another kind. This is doubtless in part due to the fact that it has been so recently brought into culture and under conditions so different from those of its natural habitat.[2] Moreover, no other of these diseases equals in possible damage the fungus under consideration. In its worst form (that is, when conditions are especially favorable to the disease) the destruction of the potato foliage by the late-blight may be complete within a few days after its appearance and that of the tubers by the asso-

[1] The studies upon which this publication is based have been in progress for a number of years. The writers wish to acknowledge their indebtedness for advice during the progress of the work to W. A. Orton and Erwin F. Smith, of the United States Department of Agriculture, as well as to William Stuart, now of the Department of Agriculture, formerly horticulturist of the Vermont Agricultural Experiment Station. Valuable assistance was rendered by W. M. Gambell in the early morphological studies; by H. A. Edson in the later cultural work, especially in the devising of new culture media; and by F. V. Rand and C. R. Orton in the determinations of disease resistance. The responsibility for the general direction of the work fell upon the senior writer. The larger part of the detailed study was made by Mr. Giddings. His removal to the West Virginia Agricultural Experiment Station early in 1909 left the later responsibility for this upon Prof. Lutman.

There are several matters upon which further study would be desirable before publication, but the removal of the senior writer to Wisconsin, following that of Mr. Giddings to West Virginia, has led to the decision to make the publication in its present form as best conducing to further progress. The authors therefore ask that it be accepted as a preliminary report of progress and hope that its lack of finality at certain points, especially as to resting-spore production and as to what constitutes disease resistance, may stimulate others to aid in the solution of the problems involved.

This report is issued simultaneously by the Vermont Agricultural Experiment Station as Bulletin No. 168 of that station.

[2] For a fuller discussion of this point, see Jones, L. R., "The Diseases of the Potato, etc.," Transactions of the Massachusetts Horticultural Society, pt. 1, 1903, pp. 144–156. Also, Jones, L. R., "Disease Resistance of Potatoes," Bulletin 87, Bureau of Plant Industry, U. S. Dept. of Agriculture, 1905.

ciated "rot" may be no less appalling. The terrible Irish famine of 1845 was due to the almost total loss of the potato crop of Ireland from this disease the preceding summer. In this country the last general outbreak was in the years 1901–1903. In 1901 Jones and Morse (1902, p. 210)[1] estimated that 65 to 95 per cent of the unsprayed potatoes on the Vermont experiment station farm showed rot following late-blight and that on August 23, 1902, the plants in two-thirds of the fields around Burlington, Vt., had died from this cause. In New York State it was estimated by Stewart of the Geneva station (Stewart, Eustace, and Sirrine, 1903, p. 252) that the loss in 1903 was 50 bushels per acre on the average, meaning a loss of nearly 20,000,000 bushels on the 400,000 acres of potatoes planted in that State that year. Morse (1909), of the Maine experiment station, estimated that 25 to 75 per cent of the crop of Aroostook County, Me., rotted in September and October, 1909, and that the loss was several hundred thousands of dollars. It was shown a half century ago that this blight and rot are caused by the fungus under consideration, but we are in ignorance as to the life history of the parasite, especially as to how it overwinters and what determines its sudden and usually unexpected outbreaks and the inception of an epidemic. The present publication aims to summarize what was previously known regarding the fungus and to record in detail the progress that has been made in the present studies toward a fuller understanding of its life history and its relations to the host. Before doing so it will be desirable for practical reasons so to describe the occurrence and appearance of the disease as to preclude its confusion with any of the other potato maladies.

CHARACTERS AND OCCURRENCE OF THE LATE-BLIGHT AND ROT CONTRASTED WITH OTHER POTATO DISEASES.

Several other diseases of the leaves and the tubers of the potato have some symptoms resembling the blight of the foliage and the rot of the tubers caused by the fungus *Phytophthora infestans*. In consequence there has been some confusion in the discussion of potato diseases even by scientific men, and discrimination on the part of the practical grower is still more difficult. As a guide to the recognition of these diseases a list of the various maladies reported for this country is here given with such a brief characterization of each as will best serve to distinguish it from the late-blight or the rot due to Phytophthora. While these helps may not suffice to enable one unfamiliar with the subject to recognize separately each of these potato maladies, it should be comparatively easy from the descriptions and

[1] Bibliographic citations in parentheses throughout the text of this bulletin refer to the "Index to literature," pp. 88–93, in which titles are arranged alphabetically by authors, separate works by the same author being placed chronologically.

the accompanying illustrations to distinguish the attacks of *Phytophthora infestans*. The disease caused by this fungus involves both the aerial and the subterranean parts of the potato plant. In this country the malady is commonly termed blight (or late-blight) as it appears on the foliage and rot (or dry-rot) as it appears in the tubers.

CHARACTERS OF THE LATE-BLIGHT AND ROT.

LATE-BLIGHT OF THE FOLIAGE.

The late-blight rarely appears on the foliage (see Pl. I) until the plants have passed the flowering stage. Indeed, it has not been observed to precede blossoming except where an early crop infects the plants of a late crop growing near by. Observations under natural conditions and inoculations also have proved that infection in this way may occur at any stage in the plant's development. At any time after the blossoming stage, if the weather conditions are favorable and the fungus is present, the disease may appear as purplish black or brownish black areas on the potato leaves. These spots are especially liable to show on the lower leaves of the plant, although one need not for this reason necessarily accept the suggestion of Clinton (1905, pp. 307–311) that there has been infection from the soil. This is, however, an interesting possibility. The diseased areas commonly appear first near the tip or margin of the leaf, although they may subsequently occur anywhere upon the leaf, petiole, or even on the stem. Infection is apparently primarily conditioned upon the accumulation of moisture from favoring dew or rain, which gives opportunity for the spores to germinate and push their germ tubes into the tissues. (Figs. 1 and 2.) If one examines blighting leaves carefully while they are still moist on a dewy morning or after a shower, a delicate growth of the fungus is perceptible as a powdery bloom on the under side, but less often on the upper, where the diseased area borders the

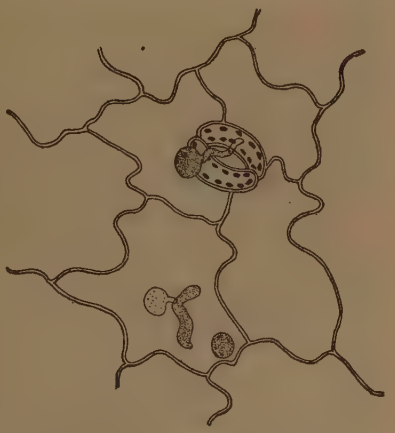

FIG. 1.—Sketch showing the penetration of a potato leaf by germ tubes of *Phytophthora infestans* through the breathing pore and the cuticle. Surface view. (After Ward.)

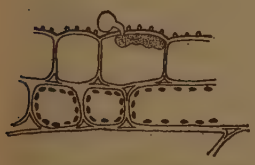

FIG. 2.—Sketch showing the penetration of the epidermal cell of a potato leaf by a Phytophthora germ tube. Sectional view. (After Ward.)

green. This aerial growth bears the spores which serve to carry the infection to healthy leaves (fig. 3).

A more detailed examination of the progress of the malady will now be worth while. In the field it does not usually attract the

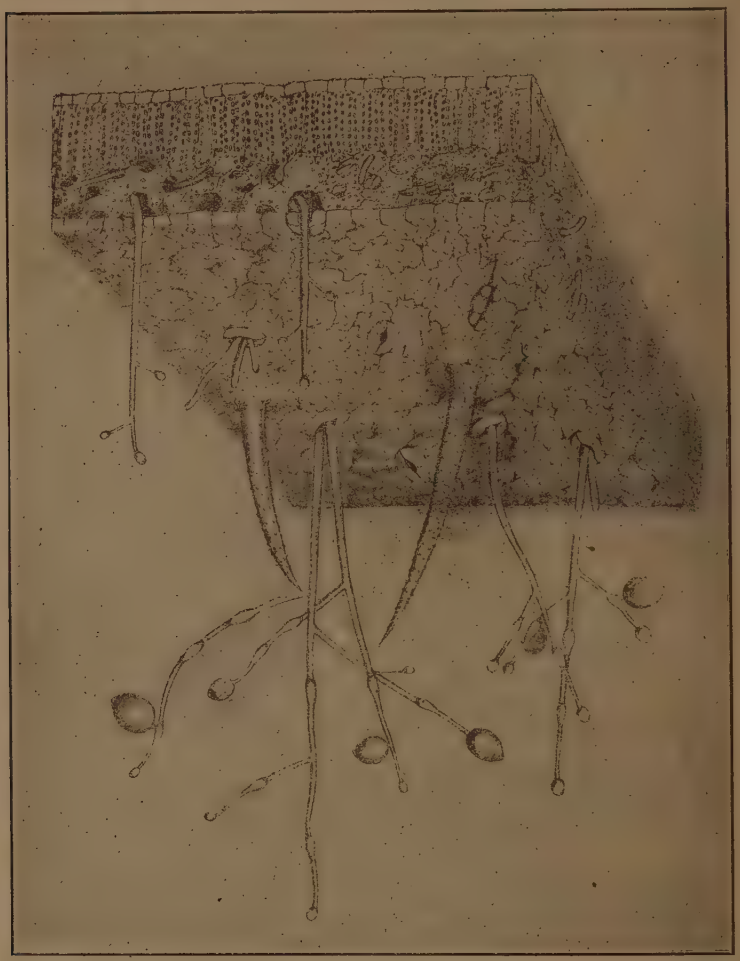

FIG. 3.—Diagrammatic representation of a square cut from a potato leaf infested with *Phytophthora infestans*, showing the fungus emerging through the breathing pores and the successive stages in the development of conidiophores and conidia.

attention of the casual observer until the upper leaves are attacked and blackened. This stage really marks the climax or even the closing stages of the epidemic and not its inception. The beginnings

antedated this by days or even weeks. The first external symptoms begin to show in about five days, on the average, from the infection of a healthy leaf, as noted by De Bary (1863) and frequently verified by the writers (Pl. I). The invaded area on the leaf takes on a water-soaked appearance, pales somewhat, then wilts and blackens. The size of the spot increases with a rapidity determined by weather conditions; if favorably moist the entire leaf will be killed in from one to four days. If the weather is dry the tissues curl up and shrivel soon after death; if moist, as is the more usual thing when the disease is prevalent, the blighted leaves become limp and soon rot, emitting a characteristic strong odor. The fact that after infection only about five days are required for the appearance of a new crop of spores explains how, when conditions best favor, it is possible within a week after the appearance of the first diseased leaves to have the foliage of all the neighboring plants stricken. As a matter of fact the malady is usually in the incubation stage two weeks or more from its early appearance upon scattered plants before it becomes generally epidemic over the field. When the epidemic stage is reached, if favored by moist weather, the entire foliage is blighted in a period of time almost incredibly short.

ROT OF THE TUBERS.

When the tops have been thus blighted (Pl. II) a varying percentage of the tubers usually show the rot, which ordinarily appears first on the upper sides of the tubers lying nearest the surface of the soil. In the earliest stages it appears as a slight brownish or purplish discoloration of the skin, and if the soil is moist the underlying tissues may be softened. In a damp, heavy soil this rotting usually spreads rapidly over the surface and proceeds inward so that the entire tuber browns and decays before harvest, constituting what is termed the "wet-rot." This is, however, as shown by Delacroix (1903) and others, largely due to the secondary invasion of such tubers by bacteria and fungi. In drier soils or seasons the progress of the disease is slower and the brown stain extends only to a depth of one-eighth to one-fourth of an inch. Meanwhile, owing to the drying out of the dead cells, the surface of the tubers wherever invaded becomes slightly sunken and of a darker color, often purplish black in the white-skinned varieties. This appearance, known as the "dry-rot," may be much in evidence at the time of harvesting, or it may show but little then and tend to develop during the first month or two of storage. If the tubers are kept in a properly cool, dry cellar this dry-rot is soon checked and they may pass the winter in this state, the superficial stain rarely extending to a greater depth in the flesh than one-fourth to one-half of an inch.

OCCURRENCE OF THE LATE-BLIGHT AND ROT.

This disease is common in the Northeastern States, especially in northern New England and New York and also in the adjacent parts of Canada. Farther south and west it is either unknown or more sporadic, unless it be on the northern Pacific coast regions. The accompanying map (fig. 4), prepared under the direction of W. A. Orton, indicates the occurrence of this fungus as based on all available records. The conclusion seems justified that the late-blight has been at one time or another introduced with seed potatoes or otherwise into practically all of the potato-growing sections of the country and that under favoring local conditions, moist weather without too great heat, it will temporarily develop anywhere. For example, the

FIG. 4.—Map of the United States showing the distribution of the Phytophthora potato disease. The sections where the disease is the more prevalent are indicated by the heavier shading. Doubtless further investigation will extend the range westward along the northern border.

most destructive epidemic of the last decade in the United States was in 1902–1904 in the northern Mississippi Valley, when the potato sections of Michigan, Wisconsin, and Minnesota were swept for two successive years by the late-blight and rot. Before that for 20 years the fungus had not been observed there and since then it has apparently disappeared again, doubtless owing to the fact that the summers are usually too dry and warm to permit its rapid spread. On the other hand, in the Northeastern States the midsummers are typically moister and cooler, and here the Phytophthora finds conditions favorable for some development almost every year and for a pro-

nounced epidemic perhaps two years out of five.[1] It seems a fair presumption that the occasional outbreaks of this disease southward and its introduction westward are the direct result of the frequent importation of the fungus along with seed tubers obtained from these northeastern regions.

OTHER DISEASES OF THE POTATO IN AMERICA.

Some of the other maladies known to occur in America from which this Phytophthora disease, or late-blight and rot, must be distinguished are early-blight, leaf-blotch, arsenical poisoning, sun scald, tip-burn, bacterial wilt and rot, black-leg, leaf-curl, Fusarium wilt and rot, scab, wart disease, internal brown-spot, and soft-rot.

Early-blight.—A widespread disease of the foliage caused by the fungus *Alternaria solani* (E. and M.) J. and G., is known as early-blight. It appears as small, irregular, sharply defined black spots on the leaves, marked by faint, target-board-like, concentric ridges. These begin to show earlier than the late-blight and in drier weather; indeed, it is rather characteristic of dry, warm soils. The progress of the disease is slow, but as the spots increase in number and size they may ultimately destroy the entire foliage, either alone or in combination with tip-burn and other maladies.

Leaf-blotch.—Another leaf-spot disease caused by a fungus, *Cercospora concors* Casp., has been observed in America (Jones and Pomeroy, 1907, pp. 236–256) in only one State (Vermont), but is widespread in northern Europe. It so closely resembles the early-blight that it may have been confused with it and occur more widely than is now known in this country. The diseased spots are larger and less sharply defined than with early-blight, without concentric markings, at first merely paler green, then turning yellow or brown and dying. The fungus is apparent as a purplish gray growth most evident on the lower surface. At certain stages it may easily be mistaken for the Phytophthora blight.

Arsenical poisoning.—The poisoning of potato leaves by Paris green or other arsenical sprays directed against insects is very common and often confused with the fungous blights. In severe cases the entire foliage is killed outright. More usually the poisoning is only local on the plant and appears as metallic-brown or blackish spots, centering as a rule about flea-beetle punctures or other breaks in the epidermis. Concentric target-board markings may occur, and often visible deposits of the poison near the center of the spot bear witness to its cause.

[1] On the relation of weather to occurrence of Phytophthora, see Ninth Annual Report of the Vermont Agricultural Experiment Station, 1895, pp. 66–71; also Bulletin 159, Vermont Agricultural Experiment Station, 1911.

Sun scald.—With potatoes, as with other tender foliage, sun scald occurs when bright, hot weather follows suddenly upon a moist, cloudy period. The scalded areas occur especially between the veins and near the extremities of the leaflets. It is commonest during the period of most rapid foliage development, i. e., before blossoming.

Tip-burn.—The name tip-burn is well applied to the drying and death of the leaves beginning at the tip and margin as a result of protracted dry heat. The dead areas blacken, crisp, and uproll. Insect or other injuries aggravate the trouble. It is commonest after the plants have passed their greatest vigor, i. e., after blossoming.

Other leaf-spot diseases.—Two types of leaf spotting occasionally occur, other than those already noted. The first is due to the slow dying and drying out of the tissues surrounding any wound in the leaf (Sturgis, 1895, p. 132). It is, in a way, comparable to tip-burn and may occur at the same time. As with tip-burn, such dead tissues are usually soon invaded by saprophytic fungi, such as *Alternaria fasciculata* and *Cladosporium* spp.

A second type of leaf spotting may occur and is sometimes quite prevalent, the cause of which is not understood. It is evidently the same malady as described by Appel and Kreitz (1907) and other European pathologists as "Dürrfleckigkeit." The blackening first appears, as a rule, along the smaller veinlets, which fact serves to distinguish the disease from early-blight; but the spotting, especially in the later stages, may occur between the veins, which makes the resemblance to typical early-blight so close that microscopic examination may be necessary to distinguish them. These spots are free from fungi in their earlier stages and are later occupied only by the common saprophytes. Appel has suggested that bacteria attacking the base of the plant may be responsible for this leaf spotting, but our own observations lead us to regard the malady as nonparasitic. This interpretation is favored by the fact that it may appear only on single plants or on certain varieties in a field without association with observable root or stem lesions and without showing a tendency to spread to neighboring healthy plants.

Brown-rot.—A disease known as brown-rot, attacking not only the potato but the tomato and other solanaceous plants, as shown by Smith (1896), is caused by bacteria (*Bacillus solanacearum* Sm.). The organisms invade the vessels of the stem, browning them and causing the base of the stem to take on a dull, muddy-green color and the foliage to wilt and die. The young tubers are also invaded, the vascular ring blackened, and a soft rot ultimately developed. If invaded stems or tubers in the earlier stages are cut across, the bacterial growth which plugs the darkened vessels may ooze out as small gray beads. This disease is most common in the Southeastern States bordering the Atlantic.

Black-leg.—A disease much more widespread than brown-rot, probably occurring in all of the older potato-growing regions, is known as black-leg. It is characterized by the rotting of the outer or cortical tissues of the lower part of the stem at or near the base. The rotting usually occurs below ground, but it may show at the surface of the soil or a little above. Plants which are attacked are checked in the early stages of growth, the leaves gradually become paler, roll upward, and ultimately die, the lower ones first. Examination shows that as a rule the seed tuber has rotted, and it is a reasonable presumption in such case that the rot started in this and spread thence to the stem base. The resulting crop of tubers may later be involved in a soft rot. Appel has shown this trouble in Europe to be due to bacteria (*Bacillus phytophthorus* Appel), and Morse (1909) and Smith (1910, pp. 748–749) have confirmed his conclusions as applicable to this disease in the eastern United States. Harrison (1906, p. 34) has described what may be the same disease in Canada as of a bacterial nature.

Potato stem-blight or rosette.—A disease having symptoms similar in some respects to black-leg has been described by Selby (1903) as rosette. It occurs in Ohio, in Colorado (Rolfs, 1904), and in several Central and Southern States. The cause is considered by these writers to be the fungus Rhizoctonia (*Corticium vagum* B. and C. var. *solani* Burt.). It is characterized by brown lesions on the underground portion of the stem and the stolons. The effect on the plant varies with the location and extent of these lesions from a dwarfed or rosette appearance of the leaves (Ohio) to a stimulation of foliage development due to prevention of tuber formation by cutting off the stolons (Colorado). The formation of numerous aerial tubers accompanies one type of this disease. This disease evidently is very similar to the black-leg type and there may have been some confusion of the two.

Potato wilt and dry end-rot.—Another widespread disease apparently most destructive south and westward in the warmer potato sections has been carefully studied by Smith and Swingle (1904), who concluded that it is caused by the fungus *Fusarium oxysporum.* The best marked symptoms are the falling or lopping over of stems and the wilting or curling of the foliage as a result of the killing of the smaller roots by this fungus. The tubers are later invaded and develop, especially in storage, a blackening of the vascular ring near the stem end. The further development of the fungus in the storage bin may lead to dry-rot, especially at the stem end of the tubers, and in advanced stages the fungus may appear as white tufts of mold on the surface.

Other leaf-curl diseases.—A form of disease characterized by leaf curling without the rapid wilting typical of the disease described by

Smith and Swingle is widespread in Europe (Blattrollkrankheit) and is probably an important factor in this country also. The causal relation of Fusarium to this disease is denied by many investigators, who believe it is partly or wholly physiological. Another type of leaf-curl malady known in Europe (Kräuselkrankheit) is occasionally seen in America. When attacked by this disease the foliage retains its normal color, but the stem, branches, and leaf veins elongate less than normal. A down curling and crinkling of the leaves results, with a denser bushy appearance of the plant as a whole. In mild cases the plant may later become normal. There is no reason to regard this as a parasitic malady. It appears to be an abnormal type of development which is, however, in its extreme form an inheritable characteristic and hence to be checked by seed selection.

Potato scab.—In all of the older potato-growing sections of America a scabbing of the tubers is common. Often deep pittings are shown, and it may even cause some cracking and may favor wet-rotting in heavy soils. In its commonest form at least this malady is due to a fungus (*Oospora scabies* Thaxter). Other forms of tuber scabbing occur, however, including the superficial black scurf caused by the fungus Rhizoctonia (Corticium) and the warty outgrowths known as the wart disease. It is probable that further study will show that still other types of scab occur.

Wart disease.—The European disease caused by the fungus *Chrysophlyctis endobiotica* Schilb. has recently been found in Newfoundland (Güssow, 1909) and is liable to be introduced elsewhere (Orton and Field, 1910). It is characterized by the appearance of warty outgrowths of the tubers which may ultimately transform the entire tuber into an irregular, deformed, black mass.

Internal brown-spot.—In the malady known as internal brown-spot the tubers outwardly appear normal, but when cut open rusty brown spots of varying size are found scattered irregularly through the flesh. These browned tissues are dead, but in typical cases are sterile, i. e., free from fungous or bacterial invasion. It is evidently a non-parasitic disease and, moreover, is not transmitted by the use of spotted seed. Probably it is due to malnutrition (Jones, 1905, p. 12).

Other causes for soft-rot.—The bacteria which cause the brown-rot and the black-leg diseases are both capable of causing soft-rot of the tubers. Soft-rot of tubers in storage or shipment from California has also been shown by Orton (1909, p. 916) to be due to a black mold (Rhizopus). If potato tubers are weakened or killed by any cause such as frost or bruising, especially if immature, and then held in storage, soft-rot may follow. Various organisms may be associated with such decay either functioning as pure saprophytes or else gaining such headway in the dead tissues that they pass on to the living tissues as facultative parasites.

EARLY OBSERVATIONS OF THE POTATO DISEASE AND ITS CAUSE.

EARLY HISTORY OF THE DISEASE.

The potato was evidently cultivated by the native people of certain parts of the American continent from prehistoric times. The earliest European explorers found it in cultivation in the temperate regions of the Andes Mountains with the appearance of having been a long-continued practice. Tubers were carried, probably from Peru or Chile, to Europe in the sixteenth century. There is some doubt as to the exact dates and places of the early importations, but the following statements, founded largely upon Roze's account (1898, pp. 61–85), are at least approximately correct. The first mention of the potato is in 1550 in a Spanish history of Peru, where it is noted that the natives near Quito cultivated it. The first clearly recorded European introduction is that into England in 1586 by Virginian colonists under the patronage of Sir Walter Raleigh, and the first account of the cultivation of the potato in Europe is by Gerarde, dating probably from the following year. There is, however, evidence that the potato was also introduced by the Spaniards into Spain, possibly at a somewhat earlier date. Roze believes this may have occurred as early as 1533. From Spain it was carried to Italy, Austria, Germany, Switzerland, and France. The first figures and detailed description of it in its new home in continental Europe are those of Clusius in 1601 in his Rariorum Plantarum Historia, although Gaspard Bauhin in his Phytopinax, printed in Basel in 1596, described it and gave it the scientific name it still bears, *Solanum tuberosum*. During the seventeenth century the potato gradually became, from a botanical curiosity cultivated only by collectors of new plants, one of the staple garden and field crops. By the latter half of the eighteenth century it was extensively cultivated and recognized as one of the regular crops throughout the temperate regions of Europe and America, so that Henry Phillips (1822, p. 78), who published in London in 1822 a detailed account of the potato and its culture, was able to cite a single grower who planted 300 acres annually. Indeed, it furnished the principal food supply of large parts of Ireland at that time.

These historical data with reference to the introduction and culture of the potato are cited in order to point out the peculiar significance of the fact that previous to 1830 there is no reference to any potato disease in Europe clearly referable to that caused by the fungus under consideration. Some time between that date and 1845 the disease appeared almost simultaneously in Europe and North America. The exact date of this initial appearance is about as uncertain as is that of the introduction of the potato itself. Some botanists, indeed, even go so far as to advance the idea that the disease was

introduced along with the potato, and thereafter was continuously present in Europe but never became general in its spread. This view seems hardly worthy of consideration, however, since it is scarcely conceivable that so destructive a disease should have long escaped definite record. The known facts all indicate rather that it was first introduced into Europe during the years 1830–1840. There can be no doubt as to the early presence of the disease in South America. De Bary (1861, p. 63), quoting from Münter's Krankheiten der Kartoffeln, says that as early as 1571 the Jesuit Joseph Acosta had observed that after damp cold weather the tubers were often destroyed in the ground "through gangrene or mildew," and Boussingault on November 17, 1845, communicated to the French Academy of Sciences a writing by Acosta in which he stated that on the high table-lands of Bogota the potatoes on low ground were everywhere destroyed in wet years.

The details as to its introduction and subsequent spread in Europe constitute one of the most interesting chapters in the history of plant pathology. We are indebted to the critical accounts of Jensen (1887), Prunet (1902), and Roze (1898, pp. 290–322) for most that follows, especially as to the spread of the malady through Europe.[1]

Hallier (1868, p. 307) cites some evidence that it may have occurred in southern Germany and invaded the Rhine Valley between 1830 and 1840. In 1842 Von Martius published an illustrated account of it as a new disease of the potato then popularly known as Stockfäule (stem-rot) and Knollenfäule (tuber-rot). Von Martius made the first scientific study of the disease and published the first description and figures, although De Bary (1861, p. 7) erroneously gives credit for the figures to Mme. Lebert, who described it in 1845 in a

[1]As already explained it was soon proved that this disease was due to the fungus *Phytophthora infestans*, which is native upon the wild potato of the Andean regions. It seems reasonable to conclude from the evidence that the outbreak of 1840–1844 followed within a few years the importation of the fungus from its native country to Europe and America. The question naturally arises as to why the disease was not transported in the earlier shipments of potatoes from South America to these countries. Evidence, to be cited later, shows that it may be carried in the tubers used for seed purposes. Jensen (1887, pp. 154–156) offers the following interesting and plausible theory:

He had already found (1887, p. 84) that the mycelium in the tubers was killed at a temperature of 25° C. The elevated Andean plateaus, where both potato and fungus are native, have so temperate a climate that this thermal death temperature is not reached. Any potato tubers carried thence to Europe in the early sailing vessels must, however, have endured long exposure to a higher temperature while passing through the tropical seas. Thus, Jensen concludes that during the first three centuries of potato culture, when the fungus was unknown in North America or Europe, the potato tubers were being disinfected by heat in the old sailing vessels while en route. After the invention of the steamship, the time spent in crossing the Tropics was much diminished. The date when those vessels came into general use, the decade 1830 to 1840, corresponds to the date of the earliest recorded occurrence of the fungus in the northern countries. These steamers could get out of the region of the Tropics in eight or nine days with large shipments of potatoes. It would take some time for the large bulk of potatoes in the hold of the vessel to be penetrated to the center by a temperature of 25° C.; and if in addition ice was used, as is common in vessels crossing the Tropics, there is no reason why the fungus might not be carried through the hot belt in this way.

periodical, "Organ des Flanders." Von Martius gave it the scientific name *Gangraena tuberum solani*. He communicated his results to the Paris Academy of Sciences and called the attention of governments and agriculturists to the gravity of the disease, but no precautions were taken. In 1844 the disease was general throughout Germany.

In France Von Martius claims the disease already existed in 1840, according to the "Echo du Monde Savante." In 1841 it was so destructive in the Pfalz, Germany, that it attracted the attention of the Royal Scientific Academy at Munich, which engaged him to study the cause of the trouble. In Belgium the disease was first noticed in 1842 in the province of Liege, and to Charles Morren (Roze, pp. 298–299), the director of the School of Agriculture, is due the first recommendation, in 1845, to use the salts of copper in combating the disease. He urged that the fields be limed with a compound consisting of 25 parts of lime, 3 of table salt, 1 of copper sulphate, and 125 of water. He says:

> This mixture is prepared in a barrel and sprayed on the surface of the ground. One ought to use this mixture for liming the potatoes even when it is somewhat troublesome. A mixture of the same composition without water should be used for disinfecting the soil. The rain will spread it in the ground and cause it to act on the germs of the disease. This liming has for its aim the complete destruction of the germs of the fungus, just as a similar liming destroys the stinking smut of wheat, the smut of oats, or the ergot of rye.

Morren's mixture is essentially Bordeaux mixture with an excess of lime. Had he conceived its use as a foliage spray instead of merely for soil treatment, this standard fungicide might have been perfected much earlier.

In Denmark the disease already existed in 1842, according to M. Fjeldstrup (1844), and by 1844 had become so prevalent that meetings were being held to discuss the calamity. In Norway the disease was known in 1841 to Westrem (1851), the director of the School of Agriculture of that country. In Ireland Cooke (1892) states that it was present in 1842 and in England in 1845, although he says in his book, "Rust, Mildew, & Mould" (2d ed., p. 145): "In all probability this potato disease was present in less striking form one or two years before it took such an alarming spread." In England the development of the disease, as recorded in the Gardeners' Chronicle, seemed to proceed from south to north in 1845, the first year when it was general and serious. The Danish journals of the same year note its appearance in Holland, then successively in the north of Germany, and finally in Denmark, showing that a similar south to north advance occurred in continental Europe. Some have inferred from this that the fungus was first introduced into the south of Europe. Jensen (1887) points out, however, that this

is the regular sequence of development which occurs annually because of climatic conditions. Plowright (1884) shows a similar condition to obtain in the British Isles. He collated reports of its appearance at the end of the month of July for the years 1877–1883, with the result that out of 100 reports from each of four sections 11 reported the disease from Scotland, 25 from the northern counties of England, 56 from the middle counties of England, and 89 from the southern counties of England.

In North America the evidence seems conclusive that the disease first appeared at about the same years as in Europe. B. M. Watson, of Boston, informed Jensen (1887, p. 151) in a letter that the disease occurred around that city in 1842; in 1843 it appeared around the city of New York (Prunet, 1902, p. 664), and in 1844 it was noted in many points in this country and Canada. In 1845 Andrew Bush, a doctor from Chester County, Pa., was awarded a prize of $20 by the New York Agricultural Society for the best essay on the rot of potatoes, at that time the disease having been present in different parts of the State of New York for at least two years. Bush's essay is worthy of more than mere mention, since his ideas were undoubtedly those of the most progressive American farmers of that time. The particular phase of the disease that impressed itself on him was the so-called "wet-rot," of whose stages he gives very careful descriptions. Apparently he did not clearly associate the blighting of the leaves with the rotting condition of the tubers, since he attributes this rot to "an epidemic condition of the atmosphere, brought into active influence by heat and moisture and producing the rot in the more tender varieties of the potato, or those raised from seed or badly cultivated, or under any circumstances unfavorable to their growth or preservation." His recommendations are to (1) plant sound potatoes from regions where the disease has not appeared; (2) give them plenty of nutrition in the form of lime, soda, potash, etc.; (3) intermix, in planting, potatoes of the same or different varieties cultivated in different soil or climate, to give the plant germinal stimulus; (4) plant whole potatoes in order to produce vigorous shoots; (5) mature the potato within 120 days of planting, i. e., plant early varieties; (6) take the potatoes up as soon as possible and store them in a cool, dry place. From this it will be seen that, while Dr. Bush had not apprehended the real cause of the disease as clearly as had Von Martius in Europe, nevertheless, his recommendations for field practice in combating it were most excellent.

The year 1845 marked the culmination of the epidemic. It swept through Europe and North America, devastating the potato fields, frequently leaving famine in its wake. In America it was most felt in Nova Scotia and New Brunswick (Farlow, 1875), where the potato

was the leading crop. In Europe none of the potato-growing sections were exempt, but the loss was most disastrous in Ireland. In 1845 over 4 per cent of all the land of Ireland was planted with potatoes and a majority of the 8,000,000 people were dependent upon them for food. There never were enough potatoes planted to last during the entire year—at best three months—June, July, and August being "meal months." When practically the entire potato crop failed, the suffering was intense. Every form of government and private aid was extended, but in spite of this about a quarter of a million people died of famine or from the fever resulting from lack of food. Since 1845 the disease has occurred in all the potato-growing countries of the world, although no subsequent epidemic has equaled that one.

EARLY INVESTIGATIONS OF THE CAUSE OF THE DISEASE.

When the disease became epidemic in 1842–1845 little exact knowledge was available concerning the relation of parasitic fungi to plant diseases. The stimulus to intensive study of plant pathology occasioned by this outbreak was enormous. Norton (1846, p. 350), writing from Edinburgh, Scotland, in 1845, says:

> To the poor (in these European countries) the potato may be considered the staff of life. In many parts thousands of families rarely obtain any other food from one year's end to another. The disease became a natural calamity. The fear of famine became universal, and every energy is aroused to avert the danger. Something must be done or the potato bids fair to become extinct. Scientific men in various countries have accordingly turned their attention to it and in most cases have been aided either by their respective governments or by agricultural societies. Among the first on the continent was a commission from Holland and Belgium. In Germany, Liebig, among others, has turned his attention to the subject. M. Payen has lately published three or four reports containing the results of elaborate microscopical and chemical researches. Bouissingault, A. Peroz, and others have also made public their opinions. In Britain and Ireland a great portion of the best scientific and practical men are now uniting their efforts. The English Government has sent to Ireland three competent chemists, with Dr. Lindlay as botanist and physiologist.

At first there was the most extreme difference of opinion as to the cause of the disease. Again quoting from Norton (p. 367) as well reflecting this state of affairs:

> Some ascribe the disease to electricity, some to atmospheric influence, some to wet season, some to wet, drought, and frost combined, some to insects or animalculæ, some to ruptures of the cell, some to decomposition of proteins.[1] others to fungi, others to a diseased and vitiated constitution in the potato weakened by long and high cultivation, others unite nearly all of the above, others still ascribe it to a direct visitation of Providence, and yet another class declare that they know nothing about it. I think these last are the safest at present.

[1] This view was held by the German chemist Liebig.

Of course, such a battery of investigations soon disclosed the fact that a fungus was associated with the disease, but the majority at first were of the opinion that this was a result or sequence of the diseased state rather than the active cause. So far as we can learn, the first scientific writer to ascribe the disease to a parasitic fungus was Von Martius (1845), whose figures of the fungus, while crude, are easily recognizable. In 1845 Morren affirmed the same idea and adduced in its favor experimental evidence based on inoculations. Moreover, Morren based his field treatment, already outlined (p. 21), on the assumption that the spores of the fungus carried the disease from plant to plant. The fungus was described by Montagne, September 3, 1845, under the name *Botrytis infestans*.

The discovery and description of the fungus were only one step, however, in the settlement of the controversy as to its causal relation. Opposed throughout to Von Martius and Morren were all the ignorance and superstition of the time, backed by such authorities as Schacht, Descaisne, and Berkeley (Prunet, 1902, p. 99). The opinion held by men like Schacht was that the fungus was only a saprophyte following closely some physiological disturbances in the leaf and probably aggravating the trouble. The opposition to the idea of the parasitism of the fungus was too great, and for a decade after the discovery of the Phytophthora it was generally regarded as incidental to the disease, not its efficient cause, and Von Martius and Morren were discredited.

Speerschneider (1857) was able to demonstrate for the first time that the spores borne on the leaves are capable of carrying the disease from them to the tubers, but as Von Holle (1858), who repeated his work the following year, was unable to get the same results the question remained unsettled. It was left for De Bary (1861, 1863) to complete the demonstration; his masterly work, which will be reviewed later, has left no doubt as to the parasitism of the fungus and its causal relation to the disease.

NOMENCLATURE AND OCCURRENCE ON OTHER HOSTS.

There was naturally some confusion concerning the nomenclature of the fungus in connection with these earlier studies. This, together with the facts as to its occurrence on other host plants, was determined by the studies of De Bary and others later. It will simplify the method of presentation, however, to summarize here the facts as to both of these matters.

The scientific naming of the fungus associated with the disease (Prunet, 1902, p. 98) is as follows:

1842. Von Martius, *Gangraena tuberum solani.*[1]
1845. Montagne, *Botrytis infestans.*[2]
1845. Lebert, *Botrytis devastatrix.*[3]
1845. Desmaxieres, *Botrytis fallax.*[4]
1846. Hartig, *Botrytis solani.*[5]
1847. Unger, *Peronospora trifurcata.*[6]
1852. Caspary, *Peronospora fintelmanni.*[7]
1855. Caspary, *Peronospora devastatrix.*[8]
1863. De Bary, *Peronospora infestans.*[9]
1876. De Bary, *Phytophthora infestans.*[10]

The scientific name for the fungus as written is accordingly *Phytophthora infestans* (Mont.) De Bary. De Bary at first followed Unger (1847) in placing the fungus in the genus Peronospora, but in connection with his special study of it for the British Agricultural Society (1875) he created the new genus Phytophthora on account of the peculiar mode of production of the conidia; several conidia, instead of one, being successively formed at the end of each branch of the treelike conidiophore, each conidium leaving behind it a swelling on the branch. No other species of similar character was then known, but six species have since been added (Wilson, 1907, pp. 387–393; Klebahn, 1909; and Coleman, 1910, pp. 620–621). The species of Phytophthora now known are as follows:

Phytophthora cactorum (Cohn and Lebert) Schröter. (*P. omnivora*, De Bary). This fungus attacks seedlings and the soft tissues of mature plants ranging through 15 families from the Pinaceæ to the Scrophulariaceæ, and of wide geographical range. A variety of this species (var. *arecae* Coleman) is a parasitic on the areca palm.

Phytophthora colocasiae Racib., a tropical fungus occurring on the taro (*Colocasia antiquorum*).

Phytophthora nicotianae Van Breda de Haan, a tobacco pest in the East Indies.

Phytophthora phaseoli Thaxter, occasionally occurring on the Lima bean (*Phaseolus lunatus*) in the eastern United States.

Phytophthora thalictri Wilson and Davis, recently discovered by Davis in Wisconsin on *Thalictrum purpurascens.*

Phytophthora syringae Klebahn, related to *P. cactorum;* occurs on lilacs, especially when stored for forcing.

Phytophthora theobromae Coleman, parasitic on *Theobroma cacao* and other plants.

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[1] Die Kartoffel-Epidemie der letzen Jahre oder die Stockfäule und Räude der Kartoffeln, Munich, 1842.
[2] L'Institut, no. 609, 1845. Société Philomathique de Paris, August, 1845.
[3] Revue Botanique de Duchatre, vol. 1, p. 151. Journal de Liege, August 19, 1845.
[4] Cryptogames de France, 1st ed., p. 492.
[5] Annales des Sciences Naturelles, Botanique, ser. 3, vol. 6, 1846.
[6] Botanische Zeitung, 1847, p. 314.
[7] Verhandlungen des Verein zur Beförderung des Gartenbaues in den königlich preussischen Staaten, 1852, p. 327.
[8] Monatsberichte der Berliner Akademie der Wissenschaften, May, 1855.
[9] Annales des Sciences Naturelles, Botanique, ser. 4, vol. 20, 1863, p. 104.
[10] Journal of Botany, 1876, p. 105.

It is also to be noted that the potato is not the only host of *Phytophthora infestans*. De Bary (1876, pp. 126, 149) mentions that it has as hosts not only a number of other species of the Solanaceæ grown in gardens, but that he has observed it on one of the exotic species of the Scrophulariaceæ, *Schizanthus grahami*, and that Berkeley has described a case where it occurred on another one of the same group, *Anthocercis viscosa*, from New Holland. It frequently happens that it becomes as destructive to the tomato as to the potato, and the first attempt in using the Bordeaux mixture against it was by Jouet (Prunet, 1902, p. 356) on the tomato. De Lagerheim (Prunet, 1902, p. 269) has found it on the edible fruits of *Solanum muricatum* at the equator, on *Solanum caripease* at Quito, and on *Petunia hybrida* at Upsala.

LATER STUDIES OF THE FUNGUS AND REMEDIAL MEASURES.

RELATIONS OF THE PARASITE TO THE HOST PLANT.

CAUSE OF THE DISEASE.

It has already been explained how it remained for De Bary (1863, pp. 59–68) fully to establish the fact that inoculation of healthy potato leaves with the spores from diseased leaves would lead to the same malady in them. It is to him, in this same publication, that we owe our first accurate knowledge of the relation of the parasite to its host plant. He found that by sowing the Phytophthora spores on the unaffected leaves the germ tubes would penetrate the epidermis, develop the mycelium intercellularly in the parenchyma, and cause the diseased spots. The manner in which the spot increased centrifugally and the transfer of the disease to the stalk and tubers were also noted by him.

The following account by De Bary (1863, pp. 46–47) will give a clear idea of his understanding of the development of the parasite and its infection of the potato:

> On the 9th of February, at 5 o'clock in the evening, the conidia were sown in drops of water on glass slides. They were then put on stems cut from the potato and placed in a damp chamber. At 7.15 the zoospores were fully developed and had pushed out a germ tube. On the morning of February 10 it was found that these germ tubes had penetrated into the potato tissue; on the 11th of February the mycelium had expanded freely in the intercellular spaces of the parenchyma, being found to a depth of six cell layers. February 14 the mycelium had gone through the whole parenchyma and numerous conidiophores were coming upon the surface of the stems. Many trials seemed to give the same results. I will cite two: Sporangia sown at noon produced zoospores at 1 o'clock. At 3 o'clock the germ tubes could already be seen buried in the wall of the host cells. February 4 conidia were sown on potato leaves; the fifth, the germ tubes had penetrated; the eighth, one of the infected leaves showed conidia; the ninth, the conidiophores appeared on the other leaves.

MICROSCOPIC STRUCTURE OF THE PARASITE.

In order to make a closer examination of the fungus it is necessary to cut thin sections from a diseased leaf and examine them under the compound microscope. It will then be seen that the nonseptate mycelial threads of the parasite occupy the intercellular spaces of the potato leaf and that through the stomata issue slender branches, the conidiophores, bearing ovoid conidia at the ends or occasionally on the sides of their smaller branches (figs. 3 and 5). It is noticeable that the walls of these conidiophores, probably in order to give them necessary stiffness, are much thicker than those of the hyphæ in the leaf and frequently have cross walls and that the side branches are swollen at places marking the points where conidia have been borne. The mycelium shows the structure common to the fungi of this group—large, nonseptate, thin-walled hyphæ, containing many nuclei (Pl. VIII, fig. 48). The conidia are borne on the aerial branches, most abundantly on the under side of the leaf; in shape they are ovoid with a short stalk and an apiculate tip (figs. 3 and 6). They are multinucleated as soon as formed and when mature contain 7 to 30 nuclei (Pl. VIII, fig. 49). The germination of this conidium may take place in either of two ways: (1) Directly, i. e., by pushing a single germ tube usually through the apiculate apex (fig. 6), or (2) indirectly, i. e., by splitting up into a variable number of biciliate zoospores (fig. 7), which, after swimming about for a short time, lose motility, withdraw the cilia, form a wall, and push out a germ tube.

FIG. 5.—Sketch showing the successive stages in the development and abstriction of the conidia of *Phytophthora infestans*. (After Ward.)

FIG. 6.—Sketch showing the direct germination of a conidium of *Phytophthora infestans* by tube formation. (After Ward.)

It was shown by De Bary (1863, pp. 42–43) that environmental conditions may determine which of these two methods of germination is followed. He was unable to determine the exact conditions for producing one or the other type, since direct germination occurred occasionally even where zoospore formation predominated. He reached the conclusion, however, that light hindered the production of zoospores. The production of zoospores occurred in

conidia when they were sown on cut potato tubers, but he found (1863, p. 41) that the other method of direct germination is the more common one in this case, although he also frequently obtained the normal production of zoospores.

This is in accord with the experience of the writers in finding that sowing the conidia in potato juice favors direct germination. It is also found that temperature affects the process. At 25° C. more than 50 per cent of the germinations are by tubes, while at 10° to 20° C. from 60 to 70 per cent produce zoospores and direct germination is exceptional. De Bary states (1876, p. 240) that each sporangium produces 3 to 8 zoospores. Although we have not made counts during germination we have found that there are more than this; indeed, De Bary's figures (1876, p. 242) would so indicate. As evidence upon this point our staining shows the conidia to have from 5 to 30

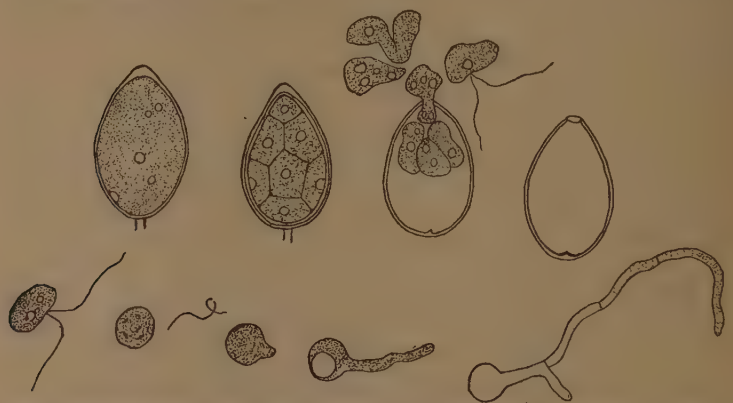

FIG. 7.—Sketch showing the indirect germination of *Phytophthora infestans* by zoospore formation. (After Ward.)

nuclei. Since the swarm spores are uninucleate they must equal this number.

While the conidia are always figured as being borne on conidiophores outside the leaf we have found that they may also be borne internally in the mesophyll. Usually these internal conidia occur singly as short side branches at any point on the mycelium, but more commonly on the hyphæ which traverse the air chambers below the stomata. These internal sporangia were apparently of the same type as the aerial ones. Their occurrence within the tissue of the leaf is of interest and of possible significance as bearing upon the nature of the resting spores discussed later in this bulletin.

The related Peronosporales typically produce sexual resting spores, oospores, and De Bary and others have searched persistently for such spores in potato leaves and tubers and in other host plants in-

vaded by *Phytophthora infestans*. It must suffice at this point to record that, although some, notably Smith (1875, 1876) and Smorawsky (1890), have stated that they found them, the evidence they presented to substantiate their claim has not been accepted as conclusive. Since our own investigations bearing on this point have been conducted almost entirely through the use of pure cultures the results will be discussed later in connection with them.

RELATION OF THE FUNGUS TO THE HOST TISSUES.

A careful examination of the relation of the fungous hyphæ to the host cells of the leaf will show that, as De Bary (1876, p. 275) noted, they press against the walls so closely that these are occasionally pushed in and sometimes penetrated. These finer structures can be best studied in properly fixed and stained sections. It will be seen then that, as Mangin (1895) has already described, this penetration of the host parenchyma cells is not a rare occurrence, as De Bary thought it to be, but that it is very frequent, particularly in the lower epidermal cells. The overshadowing effect of De Bary's work has led subsequent mycologists in general to assume that since he was unable to discover any true haustoria in the leaves there are none. It must be remembered, however, that he depended entirely upon free-hand, unstained razor sections, whereas, properly stained microtome sections will confirm the statement of Mangin (1895) that many of the parenchyma cells are penetrated by slender branches, which undoubtedly serve as haustoria. The appearance of such branches constitutes good presumptive evidence that they function actively as special absorbing organs, and this idea is further supported by the rapid collapse and death of the potato parenchyma following the fungous attack.

Very little can be made out of the relation of the haustorium to the potato cell, on account of its small size, but, as happens in many other species, such as the Erysiphaceæ, the host cell wall is extended down so as partly to inclose the parasitic branches. The fungus apparently soon dies in the black portion of the spot where the host cells are all dead, unless it be kept moist, but it continues to grow and to fruit on those that have the chromatophores largely disorganized. This ability to live in a partly saprophytic stage, while probably of limited duration in the normal course of development of the fungus, is probably the same that enables it to grow on the various culture media to be described later. The condition of the host cells is also interesting at this time. The fungus does not disorganize the chlorophyll bodies at once, but cells that are completely surrounded by the fungous mycelium still show chloroplasts full of starch grains. It is not certain as to just the meaning of this, which

is a not infrequent result of fungous parasitism. The leaf cells may be able to continue the manufacture of starch even in the presence of the parasite, or it may be that the attack is so sudden that the cell is caught with its chloroplasts full of starch and is unable to secrete the necessary diastase to translocate it.

The relation of the fungus to the cells of the potato tuber was also given some attention by De Bary (1876, p. 250), who found that the mycelium here, as in the leaf, lay largely between the cells, but that small branches, which he called suckers, were pushed into their interior. Delacroix (1903, pp. 360–362) in later years described what he considered true haustoria in the infected tissue of the potato tuber. When we had perfected the methods of handling the fungus in pure cultures on potato blocks, as described on a later page, attention was turned to this question. The Delacroix material, of which he courteously gave us a slide for examination, was taken from naturally infected, decaying tubers. For our studies thin slices were cut and sectioned from potato blocks on which the fungus was growing in pure culture. The sections were cut from the blocks at right angles to the surface, immersed in a 5 per cent solution of caustic potash to clear the starch, rinsed in water, further clarified by immersion in eau de Javelle, again rinsed, and then stained for a short time in a 1 per cent lactic-acid solution of cotton blue, as advised by Delacroix.[1]

If properly manipulated this solution gives a faint differential stain to the mycelium. Our examination fully confirms Delacroix's statement as to the occurrence of the haustoriumlike branches penetrating the tuber cells (Pl. VIII, fig. 53). It seems a reasonable inference that these haustoria contribute to the disorganization of the protoplast both by the action of toxic or enzymic secretion and by the ultimate absorption of some part of the cellular contents. The actual rotting of the tuber is due, however, as Delacroix (1903, pp. 365–373) has shown, to other fungi and particularly to the bacteria which follow the Phytophthora closely and cause the soft-rot that develops in wet soils. This is, perhaps, most convincingly shown where the fungus is grown in pure culture on sterile raw-potato tissues in the method explained later in this bulletin. Such potato tissue is never softened.

MODE OF TUBER INFECTION.

The mode of infection of the tubers in the soil is a phase of the disease that was carefully studied first by De Bary and later by

[1] The details of the method are that after clearing the starch by the potash solution and rinsing in water they were allowed to remain in eau de Javelle 10 minutes. They were then transferred, after washing in water, to a staining solution made up as follows: 1 c. c. of a saturated aqueous solution of cotton blue was added to 9 c. c. of water, then 3 or 4 drops of this diluted solution were transferred to 10 c. c. of a 1 per cent solution of lactic acid, in which the sections were allowed to remain overnight. The sections were then washed in water and mounted in glycerin.

Jensen (1887). De Bary showed that such tuber infection does not occur, as might naturally be expected, by the downward growth of the mycelium from infected leaves through the stem to the tuber. He satisfied himself that it came instead from the spores falling or being washed from the leaves into the soil. Appreciating the bearing of this method of infection upon possible remedial measures, Jensen (1887) conducted painstaking experiments to determine the relation of soil texture and depth to such infection of tubers. He states that when spores in water were poured upon a soil filter he found that less than 1 per cent of them were able to penetrate to a depth of 5.2 cm. (2 inches) and that none of them, or at most very few, were able to penetrate to a depth of 13 cm. (5 inches). Moreover, light, sandy soils held the spores much better than heavy clay.

The following experiment, recently made, gives evidence of the correctness of Jensen's general conclusion, although it shows that he relied too much on his soil filtration. Two similar trenches were dug, side by side, in a sandy garden soil. Each trench was 40 inches long by 15 inches wide and of depths varying from 4 inches at one end to 10 inches at the other. Each trench was then divided into three equal parts by upright boards set crosswise and 23 healthy Green Mountain potato tubers were buried in each compartment. In one trench (A) clay soil was used for burying the tubers and in the other (B) a light, sandy soil. In each trench the upper surfaces of the tubers were covered to a depth of one-half inch in the shallow compartment, of 3 inches in the middle compartment, and of 6 inches in the deepest compartment. Branches cut from blighting potato plants, with an abundance of Phytophthora in fruit, were then laid upon the surface of each trench and sufficient water sprayed on them to wet down the soil thoroughly. A piece of sacking was then placed over the soil and, as the weather was dry, the soil was again sprayed on each of the two succeeding days to insure an abundance of moisture such as is most favorable under natural conditions for the development of the rot. At the end of 15 days the trenches were opened and the tubers compared as to infection by the fungus, with results as shown in Table I.

TABLE I.—*Experiment showing comparative Phytophthora infection of potato tubers through varying depths of clay and sand.*

Depth of covering.	Tubers infected.			
	Trench A, clay.		Trench B, sand.	
	Number.	Per cent.	Number.	Per cent.
One-half inch (1.25 centimeters)	23	100	13	57
Three inches (7.5 centimeters)	22	96	11	48
Six inches (15 centimeters)	14	61	7	30

JENSEN'S REMEDIAL MEASURES.

Deep burying of the tubers.—As a result of his experiments Jensen devised a method of culture designed to protect the tubers from primary infection by deep burying during late summer. This he was able to do by heaping up the soil in ridges with steeply sloping sides until the vines were partly covered and the tubers were buried to such a depth that there would be no danger that the zoospores could get through the overlying soil. Assuming that the potato rows were 78 cm. (31 inches) apart, he recommends an addition of a layer of 10 to 12 cm. (4 to 5 inches) in thickness above the tubers, although, he says, a layer of 7 to 8 cm. (2½ to 3 inches) suffices in light soil, making a hill 28 to 30 cm. (11 to 12 inches) high from the bottom of the furrow. It is recommended that this be done about 10 days before flowering. In this manner the tubers could be kept sound, even though the vines were devastated by the fungus.

This method of banking up the rows very high, while successful in preventing the disease on small trial plats, was later proved to be impracticable, since it caused a considerable loss in yield. This is probably due, as pointed out by Peterman (Prunet, 1902, pp. 355–356), who tried the Jensen method at the agricultural station at Gembloux, to the strongly inclined sides of the rows shedding most of the water during rainfall and during dry weather increasing the evaporation by their slopes. In this way the tubers do not get sufficient water for their proper development and the yield is diminished. It is a method that can be successfully employed only in rainy seasons or in a very humid climate.

Tuber disinfection.—Jensen also conducted extensive experiments on the care and disinfection of tubers already invaded by the fungus, the results of which are of possible importance to the practical man as well as to the mycologist. These experiments were based largely on the facts he learned as to the thermal death point and the optimum conditions of temperature and moisture for the fungus. The death point for the spores was found to be about 25° C., while the mycelium in the tuber was all killed by an exposure of the tuber to a temperature of 30° C. for 65 hours, 35° C. for 16 hours, or 40° C. for 4 hours. The fungus, on the other hand, would not produce spores at or below a temperature of 5° C., moisture being required, of course. As a result of these observations he recommended that tubers suspected of harboring the fungus be stored in dry cellars in which the temperature did not rise above 5° C., since this will suffice to hold the disease in check. For purposes of disinfection he further recommended that potatoes designed for use as seed be subjected to a temperature of 40° C. for 4 hours in a water-jacketed chamber. So long as the tubers are not in contact with the water, this tempera-

ture, although considered sufficient to insure the destruction of the fungus within the tubers, was found not to injure the germination of the potato itself. It would seem that so simple a method as this for seed disinfection would have found immediate favor in practice, especially in the potato-growing sections of Europe, which suffer so regularly from this disease. As a matter of fact, however, its use never passed the experimental stage so far as we can learn. The chief reason for this is probably because no one has demonstrated by field experiment that such disinfection pays. Trials, though on rather a small scale, made at the Vermont station failed to show any practical benefit, and this experience seems to have been general. The further explanation of this failure probably lies chiefly in the fact that unless such disinfection were to be practiced by all growers simultaneously over a large area it would do little good, since with favoring weather conditions the fungus would spread very rapidly over a wide territory from any untreated field remaining as a center of infection. Moreover, it often seems difficult to account for the sudden reappearances of the disease at remote points and after rather long intervals if it is wholly dependent upon the vegetative mycelium in the infected tubers for its perpetuation. The possibility, if not probability, of some form of resting spore has been recognized by every student of this disease from the time of Berkeley and De Bary.

STUDIES OF INFECTION AND DISSEMINATION MADE IN VERMONT.

PRACTICAL QUESTIONS INVOLVED.

Although Jensen's method for tuber disinfection has not come into practical use, the questions involved, especially as to time and method of tuber infection in the soil, the spread of that infection to other tubers either in the soil or in the storage cellar, and the production of infected plants from infected seed tubers, are all matters of so much economic importance that further investigations bearing upon them have been undertaken from time to time in connection with the Phytophthora studies at the Vermont station. The current ideas on the subject, based largely upon De Bary's work, are as follows: The conidia falling from the diseased leaves to the ground infect the tubers under them, producing a primary infection; from this primary infection the fungus may spread from one tuber to another, at least in the storage cellar; tubers may be quickly killed by the rot or they may be invaded by the fungus without showing evident external symptoms of the rot and, if such are planted the following season, the shoots coming from them may harbor the fungus, which will in due season sporulate, thus starting the infection of the second year.

In the work that has been done at this station in connection with the present studies, the attempt has been made to secure further evidence along these lines upon several matters of more or less practical importance.

1. When and how does the primary infection of the tubers occur in the field?

2. Does secondary infection of the tubers occur in the field before digging, i. e., may the fungus spread from tuber to tuber in the soil?

3. Are healthy tubers liable to infection from contact with blighting foliage at the time of digging?

4. When the blight appears on the foliage should the crop be harvested at once or should the digging of the tubers be delayed until the season of normal maturity?

5. May infection occur in storage, i. e., may the fungus fruit on mature tubers in storage and the spores infect other mature tubers?

6. If such infection does occur is it through the unbroken skin, the eyes, or the lenticels?

7. Do infected seed tubers give infected plants if used for seed?

PRIMARY INFECTION OF TUBERS IN THE FIELD.

De Bary, Jensen, and others concluded as a result of their studies that the tubers are infected, as a rule at least, by spores washed through the soil from the blighting foliage. It has been repeatedly observed when digging potatoes that tubers at or near the surface show more rot than those buried deeper in the soil and that the rot is more apt to begin on the upper side of the tuber. Examinations have shown that it appears nearly as often at the tip end of the tuber as at the stem end. All of these facts are in accord with the above idea that the infection passes from the foliage through the soil rather than by way of the stem. Moreover, experiments which will be summarized later were made at the outset of the work (Jones, 1891, 1893) which showed that by properly spraying the foliage the development of the fungus is checked and that when this is done little or no rot develops in the tubers. This also is in accord with the idea outlined above so far as it bears upon the question. In order to meet the conditions of the problem more directly, trials were made to learn what will be the result if the surface of the soil is sprayed while the foliage is unprotected and attacked by the fungus. This is, indeed, essentially what Morren (1845) recommended some 65 years ago as a means of checking the rot of the tubers. Opportunity was offered for trial of this method during two seasons, 1902 and 1905. In each case the blight was prevalent and all the foliage of the trial plats was attacked and destroyed.

In the first trial (Jones and Morse, 1902) the soil was sprayed twice, August 13 and September 6. The second trial (Jones and

Morse, 1905, p. 273) involved three sprayings, August 2, 18, and 28. The results are shown in Table II.

TABLE II.—*Comparative effect of spraying and nonspraying of soil for control of rot of potato tubers.*

Treatment of soil.	Percentage of rot.	
	Experiment 1.	Experiment 2.
Not sprayed	19	81
Sprayed	2	17

A marked reduction in the percentage of rot in the sprayed soil is shown for both seasons, this reduction being in accord with the idea that tuber infection occurs primarily through the soil. In neither case were the experimental conditions perfect. In the first experiment some development of the fungus on the foliage had occurred, and so also possibly some tuber infection, before spraying began. In the second experiment a heavy rainfall occurred in September when the fungus was rampant on the foliage, and the tubers were not dug until October 13. Doubtless one or more additional sprayings of the soil in September and early October would have further reduced the rot.

SECONDARY INFECTION OF TUBERS IN FIELD OR STORAGE.

The possible importance of secondary infection, i. e., the passage of the fungus from tuber to tuber in the field or storage bin, was emphasized by observations made in the autumn of 1905. When the crop growing in a moist, heavy soil was dug, considerable rot was found and a large number of the tubers which were in the early stages of decay had small white tufts of the Phytophthora (Jones and Morse, 1905, pp. 284–287) scattered over the surface and bearing abundant crops of spores capable of germination (Pl. III, fig. a). Moreover, in some places the mycelium was ramifying through the interstices of the soil at least one-half an inch from the surface of the decaying tuber, although doubtless nourished by it. All the tubers showing decay were sorted from the crop, which was then stored in shallow boxes in a dry cellar at 50° to 55° F. Later, in October, these boxes were reexamined and occasional tubers were again found bearing thrifty tufts of Phytophthora, rupturing the lenticels and sporulating abundantly (Pl. III, fig. b). It is evident, therefore, that the fungus may fruit freely enough on the tubers, either in field or storage, to supply spores for further tuber infection, providing mature tubers are susceptible.

Repeated trials have shown that sowings of the fungous spores on mature potato tubers at any time during their dormant period will lead to infection if sufficient moisture is present. The results of a

single experiment are here given. Twenty-four healthy Green Mountain potatoes were selected in midwinter and arranged in groups of six. Conidia from a vigorous culture of Phytophthora were left in water until they began to germinate. A drop of this spore-laden water was then placed upon each tuber: In the first group in an eye of each tuber; in the second, upon a prominent lenticel; in the third, upon a spot slightly abraded so as to expose the flesh; and in the fourth, upon the unbroken skin where free from visible lenticels. Each point of infection was marked by an India-ink circle and the tubers were then stored in a moist chamber and kept at about 18° to 20° C. At the end of three weeks all of the tubers inoculated in abrasions showed infection. Two of those having spores applied to the eyes, two having spores on lenticels, and one having spores on the unbroken surface were infected. From this experiment it is clear that the mature tuber is not proof against infection even where no bruises or wounds occur, although freshly bruised or wounded tubers are more readily invaded.

INFECTION OF TUBERS AT TIME OF HARVEST.

The results outlined suffice to show the danger of infecting the tubers by harvesting the crop at a time when the partially blighted tops are heavily laden with spores. A trial made in 1905 (Jones and Morse, 1905, pp. 285, 286) bears still more directly upon this point. The freshly dug tubers from a blighting field were sorted into two piles; one was covered with a layer of the blighting tops, the second with sackcloth. Both piles were then sprinkled with water and allowed to remain overnight before putting them in the storage cellar. The result was that every tuber from the first lot decayed within eight weeks, while only 54 per cent of the second lot developed rot. Of course there had been abundant infection in the field preceding digging, but the infection from the blighting tops was so complete that it doubtless would have destroyed all of the tubers of the first lot had there been no field infection.

RELATION OF DATE OF DIGGING TO DEVELOPMENT OF ROT.

Several of these facts have a bearing upon the question of the relation of the date of digging to the development of the rot. The fact that the infection washes from blighting tops through soil to tuber argues for early digging when the disease is bad. Opposed to this is the fact that digging while the blighting tops are producing spores may expose the tubers still more generally to infection and that the disease may spread more rapidly in the freshly bruised immature tubers. Experiments were carried on during two seasons (Jones and Morse, 1902–3, 1903–4) which gave convincing evidence that under any except the most unfavorable soil conditions early digging is unwise. The average of the trials of two seasons (Jones and

Morse, 1903–4, pp. 391–395), including tests upon several different fields and varieties, was as shown in Table III.

TABLE III.—*Experiments showing relation of date of digging of potatoes to development of rot.*

Date of digging.	Sound potatoes dug.	Development of rot.	Date of digging.	Sound potatoes dug.	Development of rot.
1903.	*Pounds.*	*Per cent.*	1904.	*Pounds.*	*Per cent.*
Aug. 31	84	55.3	Aug. 22	403	4.3
Sept. 7	150	25.7	Aug. 29	440	11.8
Sept. 14	169	15.8	Sept. 5	510	18.4
Sept. 21	175	11.4	Sept. 12	599	8.9
Sept. 28	171	7.3	Sept. 19	578	8.0
			Sept. 26	618	6.0

The results of these experiments led to the formulation of the general rule that where the tops are attacked by the late-blight the harvesting of the tubers should be delayed until a week or more after the death of the tops and that longer delay does no harm, but in exceptionally wet seasons and on low, heavy soil, where the fungus develops rapidly on the tubers underground and so spreads in the hill, early digging is necessary, else the entire crop may be lost.

RELATION OF STORAGE CONDITIONS TO DEVELOPMENT OF ROT.

The relation of storage conditions to the development of rot has also received practical test in connection with these studies. While it has already been shown that the fungus may, under exceptionally favorable conditions, continue to fruit on the tubers in storage and so spread from tuber to tuber, our trials lead us to believe that if the cellar is properly dry and cool this will not occur to any serious degree. The use of lime and disinfection with formalin have proved to be valueless under such circumstances (Jones and Morse, 1905, pp. 280–281). On the other hand, the drying of tubers before storage (Jones and Morse, 1905, p. 282) and also the low temperature of the storage cellar, as advocated by Jensen, are important. Thus the apparently sound tubers from a field where the tops were killed by the late-blight were divided into three lots and stored at about 40°, 55°, and 70° F., respectively. The amount of rot at the end of two months was 17, 53, and 79 per cent, in the order named.

PROPAGATION FROM SEASON TO SEASON.

De Bary (1876, p. 267) followed the fungus from infected seed tubers into the stem and foliage of the shoot developing therefrom and observed the sporulation upon these aerial parts. There can be no doubt, therefore, as to the possibility of the fungus overwintering in this way, and it seems probable that this method is the common means of propagation from season to season. De Bary (1876, pp. 266–269) first experimentally tested this matter in the field by planting artificially inoculated tubers and found that while no sprouts

would come from the eyes in or near the diseased areas they would come from others at some distance away, some of these latter ones being affected by Phytophthora. Jensen (1887, pp. 120–122) repeated the experiments in 1883 by planting diseased and healthy tubers in parallel rows, at the same time attempting to discover whether the diseased shoots would be able to reach the surface of the ground in case the diseased tubers were planted deep. Fifty diseased tubers were planted 10 centimeters deep and a like number 21 centimeters deep. From those at a depth of 10 centimeters 24 plants came up; of these 24 plants only 1 was very vigorous, 18 were unaffected by the disease, while the other 6 showed it as brownish spots on the stem near the ground. Of the 50 diseased tubers planted 21 centimeters deep only 19 came up, and 1 of these was diseased and soon died. From this experiment he concluded that the deeper planting made it impossible for the weakly, diseased shoots to pierce the thick layer of soil on top of them and that there must be a delicate balance among these three factors—the vigor of the shoot, the virulence of the Phytophthora, and the thickness of the layer of soil the shoot has to penetrate before reaching the light.

In our earlier experiments attempts were repeatedly made to get evidence bearing upon this overwintering of the fungus by planting tubers showing varying degrees of dry-rot which was started by natural infection with Phytophthora. In no case, however, has positive evidence been secured. Either there was no growth or else the sprouts appeared normal. So far as conclusions are justified by such negative results, it would seem that if the fungus successfully overwinters in the tubers it is in cases where its attack is very mild, i. e., where it remains so nearly dormant that it causes little or no dry-rot or other visible injury. In two later experiments artificial infection has been resorted to in order to learn the relation of the fungous invasion to the development of the shoots. On May 21, 40 healthy Green Mountain tubers of uniform appearance and with strong sprouts one-half inch to 3 inches long were chosen for the experiment. Twenty of these tubers were inoculated with bits of mycelium from pure cultures in slits cut near two or three of the healthy eyes in each tuber, these eyes being marked by India ink. The other 20 tubers were left uninoculated as controls. All of the tubers were held in a moist chamber for two days in order to give the fungus a chance to penetrate the tissues. All of the uninoculated eyes were then cut out of the inoculated tubers and all except two or three from each of the control tubers; they were then planted. The control tubers all grew, sending up 37 shoots, whereas only 5 shoots appeared from the inoculated tubers. All of the shoots appeared normal and remained healthy.

On May 22, 60 healthy Early Rose tubers of approximately the same size and with sprouts one-half inch to 1½ inches long were chosen for trial planting, 30 to serve as control and 30 to be inoculated. Of the inoculated tubers, 4 were treated by placing a drop of water containing the spores in the depression of a healthy uninjured eye, 16 were treated by placing the spore-bearing drop in an incision near an eye, and 10 had a bit of mycelium placed in a similar incision. The inoculated spots were marked as in the previous experiment and the tubers similarly treated, i. e., held two days in a moist chamber, the extra eyes removed, and the tubers then planted. The results were as shown in Table IV.

TABLE IV.—*Phytophthora inoculation of Early Rose tubers.*

Treatment given.	Number of tubers.	Shoots.	
		Number.	Description.
Spores in uninjured eye	4	0	
Spores in incision near eye	16	3	Weak.
Mycelium in incision near eye	10	3	Do.
Control (not inoculated)	30	51	Strong.

All of the shoots from the inoculated tubers were weak and died within a short time, whereas the shoots from the control tubers matured normally and no trace of Phytophthora was detected on any of them. This is not surprising even though the fungus were present internally in the shoots from the inoculated tubers, since dry and hot weather continued during early summer and until after the death of the weak shoots from the inoculated tubers. Watering and partial shade were tried in the hope of inducing sporulation, but without success. These results, while not convincing in themselves, are in accord with Jensen's conclusion that when the young shoots are invaded by the fungus they are thereby weakened and may be killed outright before reaching the surface of the soil. The probability is that if the fungus overwinters as mycelium in tubers used for seed the first sporulation of the second season will appear on weak shoots which rise but a short distance above the surface of the soil and that from these shoots the spores will be scattered to the lower leaves of the overshadowing healthy plants. It is also to be noted, however, that much the same stages may occur in case the initial infection of the young shoots comes from the overwintering of a resting spore.

MODES OF DISSEMINATION IN THE FIELD.

The manner in which the rapid dissemination of the fungus is brought about following such primary infection is important as helping to determine what conditions favor the development of an epidemic. Moisture is, of course, essential for spore germination, and it is noteworthy that sporulation occurs abundantly only in a

moist atmosphere. Probably the washing and dashing about by rain is the most effective agency for local dissemination. For longer distances two agencies probably cooperate, wind and insects. The former is so constantly operative that, while most spores carried by it must certainly perish by desiccation, enough may survive to carry the infection to distant plants.

The importance of insects in the dissemination of plant parasites has been clearly presented by Smith (1896) and others. In order to make a specific test of their ability so to function in the case of this fungus, the following observations were undertaken.

On the morning of July 15, 40 larvæ or slugs of the Colorado potato beetle were placed on Phytophthora-bearing potato leaves under a bell jar. That night the larvæ were transferred to two healthy potato plants, which were then covered by a bell jar until the next morning. The insects were then killed. The plants, which were growing in pots, were kept well removed from any other potato plants. After six days diseased areas appeared upon two leaves of one plant and one leaf of the other. The mature beetles by their more active habits and their migration on the wing are, of course, capable of carrying the Phytophthora spores to greater distances than the larvæ and doubtless are important agents in the spread of the fungus in potato fields, especially in the earlier stages of its development.

SPRAYING THE FOLIAGE A PRACTICAL REMEDY.

No plant disease has received so persistent and widespread attention as this in the search for effective remedies. The only measures which have given much return, or, indeed, which give large assurance of such to date, are foliage spraying and the development of disease-resistant varieties. Both of these measures have been so fully dealt with in previous publications (Jones, 1899, 1905; Stuart, 1906) that only the briefest summary is justifiable here.

Attention has already been called to the early recommendation of Morren (1845) of a mixture of copper sulphate, salt, and lime as a remedy to be sprinkled on the soil. Had he tested this on the foliage greater results might have followed. Since then practically every fungicide of promise has been tested by one or another experimenter upon potatoes. Freshly prepared Bordeaux mixture holds its place, however, as distinctly the best remedy. When rightly prepared and intelligently used it is a remarkably effective potato fungicide. The credit for the first demonstration of its value in controlling Phytophthora belongs to M. Jouet, who in 1885 used it on tomatoes in France. In 1888 Prillieux used it effectively on potatoes. He prepared a mixture containing 6 parts each of copper sulphate and lime in 100 parts of water which he applied to the potato foliage when

the fungus made its first appearance in the field with apparent benefit, although in spite of it he lost about 25 per cent of his yield from rot. During the next three years, trials of this promising new remedy were made at several of the recently organized American experiment stations as well as in European countries other than France. Sorauer (1893) in Germany found that by spraying with Bordeaux mixture after each rain he could save all but 1 to 2 per cent of the tubers. Extensive experiments in England and Scotland under the auspices of the Royal Agricultural Society confirmed the results obtained in France and Germany. The most pronounced benefits from the experimental use of this mixture, and the most extensive applications in practice, have obtained in America. Probably this is in part due to the fact that this country has a variety of potato pests, especially the Colorado beetle, whose ravages have forced the progressive potato grower to use some kind of spray, and the Bordeaux mixture in combination with arsenical poisons has proved a remarkably effective panacea for the numerous other potato pests, insect and fungous, as well as for Phytophthora. At the Vermont experiment station the first trial of this mixture for the potato fungus was made in 1889 (Minott, 1890), and annual trials of spraying late or main-crop potatoes two or three times with Bordeaux mixture were continued for 20 years consecutively. A detailed review of this work is given by Lutman (1910). The averages from these trials are shown in Table V.

TABLE V.—*Averages of 20 years' trials in Vermont, showing gains from the use of Bordeaux mixture on late potatoes.*

Variety planted and date of planting.	Dates of spraying.	Yield per acre.		Gain per acre.	
		Sprayed.	Not sprayed.		
White Star:		*Bushels.*	*Bushels.*	*Bushels.*	*Per cent.*
May—, 1891	Aug. 26, Sept. 8	313	248	65	26
May 20, 1892	July 30, Aug. 13, 25	291	99	132	194
May 20, 1893	Aug. 1, 16, 29	338	114	224	196
Apr. 26, 1894	June 16, July 17, Aug. 30	323	251	72	29
May 20, 1895	July 25, Aug. 13, 31	389	219	170	78
Polaris:					
May 15, 1896	Aug. 7, 21	325	267	68	26
June 1, 1897	July 27, Aug. 17, 28	151	80	71	89
White Star:					
May 10, 1898	July 21, Aug. 10	238	112	126	112
Three varieties (average):					
May 18, 1899	July 26, Aug. 17, Sept. 8	229	161	68	42
Delaware:					
May 23, 1900	Aug. 4, 23	285	225	60	27
May 25, 1901	July 20, Aug. 21	170	54	116	215
May 15, 1902	Aug. 1, 20	298	164	134	82
Green Mountain:					
May 1, 1903	Aug. 10	361	237	124	52
Delaware:					
May 25, 1904	Aug. 1, Sept. 1	327	193	134	69
May 15, 1905	Aug. 2, 21	382	221	161	73
Green Mountain:					
May 27, 1906	Aug. 13, 22	133	101	32	32
May 1, 1907	July 16, 25, Aug. 8, 22	171	63	108	175
May 15, 1908	June 26, July 9, Aug. 6, 26	156	65	91	140
May 28, 1909	July 12, 23, Aug. 6, 27	243	188	55	29
May 9, 1910	July 11, 27, Aug. 15, 23, 30	240	202	38	18
Average for 20 years		268	163	105	64

The average gain for the 20 years of spraying was 105 bushels per acre. The greatest gain was in 1893, when three applications made in August increased the yield of marketable potatoes from 114 bushels to 338 bushels per acre, or nearly threefold. The loss that year on the unsprayed plat was almost wholly due to Phytophthora. In all places where Bordeaux mixture is used in the Northeastern States some gain is attributable to its beneficial action against other potato pests, especially the early-blight and the flea beetle, as well as to the indirect stimulating effect which it exercises. Similar evidence as to the profitableness of the regular use of this spray on potatoes has recently been furnished by the trials of Stewart, of New York, and Morse, of Maine. Stewart, French, McMurran, and Sirrine (1910) had an average increase of 102 bushels per acre during the seven years (1902–1909) they have experimented with potato spraying at the Geneva (N. Y.) station. Morse (Bul. 169, 1909) was able to reduce the rot from the 25 to 75 per cent occurring on unsprayed fields to 0.6 to 0.9 per cent where the spraying had been thorough. It is evident, therefore, that by the intelligent use of Bordeaux mixture both the foliage and the tubers of the potato can be practically insured against serious attacks from Phytophthora even when it is epidemic. The cost of this is small in proportion to the gain. Stewart (Stewart, French, McMurran, and Sirrine, 1910, p. 44), in farm practice in New York, found the average cost for seven years to be $4.82 per acre for the season. Nevertheless, the potato grower has the right to expect at least some measure of relief from this necessity for spraying. This relief may be expected in part from the development of more highly disease-resistant varieties and in part from the completion of our knowledge of the life history of the fungus. Work like that now in progress on the life history in time should remove the last vestige of the breastwork which as yet obscures the location of the foe during certain seasons. The remaining pages of this bulletin contain the records of some attempts to further our knowledge along both of these lines.

GROWTH OF THE FUNGUS IN PURE CULTURE.

EARLIER TRIALS IN EUROPE.

In the earlier studies of *Phytophthora infestans*, 1860 to 1875, of course no attempt was made to get it into pure culture, since such a thing was unknown at that time. The only aim during that period was to get it and keep it comparatively free for a short time from contamination by other fungi. The growth of the fungus on the leaves and aerial parts of the plant had been repeatedly observed since its discovery by Von Martius and the description of the species in

1845 by Montagne. De Bary (1863, p. 94) was apparently the first to obtain the fungus with anything like a pure growth of mycelium and conidiophores on the tubers. His method, which has become the classical one for demonstrating the presence and methods of fruiting of the fungus, consists in cutting open an infected tuber and keeping it under a bell jar where the surface will continue damp. In a few days, under favorable conditions, a growth of Phytophthora will appear on such surfaces and the mycelium and fruiting branches can be obtained in a fairly pure state. De Bary (1863, p. 64) was able to transfer the conidia either to the uninjured tuber or to the cut surface of other uninfected potatoes and cause infection.

Since the fungus has been generally regarded as a strict parasite, without any saprophytic phase in its life history, it has been commonly considered impossible to induce it to grow on anything but the living potato or potato plant. Recent work, however, has shown that this conception is an erroneous one and that, while it is strongly parasitic in its habits, it is not an obligate parasite. The range of its saprophytism is rather narrow, however, as will appear later.

The earliest attempt at growing the fungus on substrata other than living potato tubers and leaves was that of Hecke (1898), who cultivated the Phytophthora on decoctions of plums, of tomatoes, of cherries, and of potato leaves, but was unable to induce it to grow on infusions thickened with gelatin. He also found that the fungus could not stand a higher concentration than 1.5 to 3 per cent of dry substance, while a still lower concentration favored its growth.

The next account of the isolation and cultivation of the potato fungus was that of Matruchot and Molliard (1903), who succeeded in growing it on solid media. They were able to transfer it from the fresh surfaces of infected potatoes kept under a bell jar, as described by De Bary, to sterile plugs of potato in test tubes. The growth and fructification obtained under these conditions were normal and abundant. Plugs of pumpkin and muskmelon (melon d'Espagne) gave practically as rapid a growth and as large a quantity of fruiting branches. The curious fact was brought out that the fungus would not grow or produce conidia on potato plugs sterilized at 115° C., while it would grow almost as well and produce as many conidia on the cooked pumpkin plugs as on the raw ones, and almost as well on the cooked muskmelon as on the raw. The cause for this difference they believe to lie in the fact that the superheated steam so swells the starch grains of the potato that the fungus finds it mechanically impossible to push its hyphæ between them. The best artificial media for its growth were found to be potato or pumpkin broths or such broths thickened with gelatin. In all these broths fructifications occurred abundantly.

MORE RECENT TRIALS IN AMERICA.

In 1904, and before the results of the preceding workers came to our attention, pure cultures of the potato fungus were obtained on sterile potato blocks in the Vermont laboratory, and since that date it has been carried continuously in pure cultures, during the earlier part of the time on potato blocks in tubes, but more recently on gelatin or agar media. Before discussing our methods and results in detail it will be helpful to review the work of Dr. G. P. Clinton, of the Connecticut station, who has meanwhile contributed some helpful facts and methods along similar lines.[1] Clinton (1906) confirmed Matruchot and Molliard's results on the cultivation of the fungus on plugs of living tissue of potato or pumpkin. He found further that the fungus would grow better on sterilized corn meal or on a mixture of ground green Lima-bean pods and seeds with corn meal than on any other of the artificial media he tested. Some growth was obtained on agar cultures to which various ingredients had been added, but on the whole they were unsatisfactory, potato and pumpkin juice being better. In a more recent paper Clinton (1908) reports upon further trials in the cultivation of the fungus. In these trials he found that the soluble substances produced a medium on which the fungus grew and fruited as well as on living plugs of the potato or pumpkin. He also tried a number of other media, among them the potato-juice gelatin in use in the Vermont laboratory, but found it not to be a very good medium because the surface layer would dry out and it was difficult to get the fungus started.

In our Vermont laboratories *Phytophthora infestans* has been grown continuously in pure cultures since 1904 by the writers and others, as stated in the introduction to this bulletin. The chief incentive at the outset was the possibility of the discovery of oospores or other resting spores. Later the aim has been, in addition, to contribute something to the knowledge of the occurrence and nature of disease resistance to this fungus, as shown by certain potato varieties. Progress toward these goals was, however, made very slow by the difficulties encountered in the perfection of culture methods and media, and perhaps the most important gains to date lie in the development of cultural methods and their application to the testing of disease resistance. In the succeeding pages the aim has been to outline these attempts in sufficient detail so that anyone wishing to repeat or continue this line of investigation may be able to profit as far as practicable from our experience. Therefore, the general methods and results of the cultural studies will be

[1] Clinton's latest results (1910), including an important new culture medium, have come to hand just as this bulletin is going to the printer. A brief review of these results is inserted on a later page.

outlined before special attention is given to the matters of resting spores and disease resistance.

DETAILS OF TRIALS MADE AT THE VERMONT STATION.

SOURCES OF THE PURE CULTURES EMPLOYED.

In connection with these studies pure cultures have been obtained from tubers from different sources, including several from Vermont, two from Connecticut (G. P. Clinton, one tuber and one culture), one each from Maine (W. J. Morse) and Pennsylvania (W. A. Orton), two from England, and one each from Holland, Germany, Ireland, and Scotland. The methods used in isolating and cultivating the fungus will be given in sufficient detail so that anyone desiring to do so may be able to repeat the work. The cultures have been secured in all cases from tubers showing the dry-rot, those in the early stages being chosen where practicable. The tuber selected is split open with a sterile knife, placed under a bell jar in a moist atmosphere to favor the development of the fruiting fungus on the freshly cut surface, and as soon as the growth appears it is transferred to sterile raw-potato cylinders prepared as described later. Bacterial contaminations often occur which necessitate several further transfers for their elimination, and this is made easier by the use of the bean-agar medium described later.

VARIOUS CULTURE MEDIA TESTED.

In the course of these studies a wide variety of culture media has been tested. Some of these media proved more or less favorable for the growth of Phytophthora, while others were entirely unsuited. Normal growth and fruiting have been obtained on raw-potato and pumpkin blocks, potato gelatin, and Lima-bean agar. On certain other media, such as pumpkin agar, pumpkin gelatin, and some of the synthetic media, there was some growth, with few or no conidia, while on many others no growth was apparent. In considering the details as to the behavior on these media it will prove helpful to group them as follows: (1) Raw and cooked vegetables; (2) vegetable broths, either alone or with gelatin or agar; (3) synthetic liquid media; (4) synthetic media with agar or gelatin; (5) synthetic liquid media and silicate jelly. All cultures were carried at 18° to 20° C. unless otherwise specified.

GROWTH ON VEGETABLE TISSUES.

The media tested in these trials included raw or living potato tubers, raw muskmelon and pumpkin rinds, parsnip roots, and also

cooked tissues from different parts of the potato and pumpkin. The raw potatoes were prepared as follows: Medium-sized sound tubers were washed, immersed 10 minutes in a 0.1 per cent solution of mercuric chlorid to disinfect, the surface layer was cut away with a knife kept sterile by repeated flaming, and blocks about 1 by 1 by 4 centimeters in size were cut from the interior. Each such block was then dropped into a sterile culture tube which contained about 1 cubic centimeter of water, either with or without absorbent cotton in the bottom. The medium was then allowed to remain at least three days to test sterility before inoculation. The other raw vegetables were prepared in essentially the same way. The cooked vegetables were prepared by using similar blocks covered to about one-third their depth with water sterilized by exposure to flowing steam on three consecutive days. Inoculations upon such raw-potato blocks cut from tubers of Early Rose, Green Mountain, or other varieties favorable for the fungus will yield a prompt growth which is in full fruit in 5 or 6 days, and by 10 days has reached its maximum, when the surface of the potato is often completely hidden under the cottony mycelium (Pl. IX). Thereafter the culture may soon die out, although the longevity of such potato cultures may be surprisingly great, especially if desiccation is checked. Thus, one such culture which had been sealed for 7 months was found to be living when opened. Where invaded by the fungus the potato tissue is usually discolored to brown or black for several cells in depth, although the degree of this discoloration varies widely with varieties and in some it is very slight. It must be that Matruchot and Molliard (1903, p. 542) used varieties of this latter type, since they observed no discoloration. In vigorous cultures, 5 to 8 days old, droplets of clear brownish liquid exudate will frequently appear upon the surface of the mycelial layer, apparently as a result of a normal bleeding process comparable to that observed in Pilobus and other fungi (Pfeffer, Eng. ed., p. 275). In order better to observe this exudate, cultures were made on large slices of potato in petri dishes. Upon these slices the exudate appeared in quantity while the underlying potato tissues were shrunken in proportion, indicating that water was being withdrawn from these tissues to supply the loss to the fungus above. Sufficient of this exudate was collected for titration with phenolphthalein. It was found to be slightly acid in reaction, but less so than the normal potato juice, showing that in passing through the fungus it is modified toward alkalinity. A comparison of blocks cut from just below the surface of the large-sized tubers with others from the center of the same failed to show any difference in the resulting growth. There may, however, be a marked difference in the growth on blocks cut from tubers of

different varieties. The consideration of this feature is given further attention under "Disease resistance of potatoes" (p. 69).

In contrast with this vigorous normal growth on raw potato is the fact that repeated trials have failed to obtain any growth on cooked potato. As already stated, Matruchot and Molliard (1903, p. 541), who had like results, attributed this immunity to the mechanical obstruction due to the swelling of the starch grains by cooking, which prevented the penetration of the fungus. Partial confirmation of this idea is found in the fact that some growth is obtained in cooked potato broth.

On raw pumpkin the growth was much slower than on potato, but ultimately it was fair with normal conidial fructification. On cooked pumpkin it was somewhat weaker, but gave a moderate crop of conidia.

GROWTH ON VEGETABLE EXTRACTS EITHER ALONE OR WITH GELATIN OR AGAR.

This phase of the cultural studies has at the same time suggested so much of promise and presented so many difficulties in development that it has received a relatively large amount of attention. Potato juice was pressed from the living potato tissues, leaf and tuber, and sterilized by passing through a Pasteur-Chamberland bougie into a sterile vessel. The leaves of both susceptible and resistant varieties were tried as a source of this juice, as well as the tubers of the same varieties. Juice was extracted both from the surface tissues and from those of the interior of the potato tuber. The results are taken up in detail under "Disease resistance of potatoes" (p. 69) and so will be only briefly summarized here. Little difference was apparent in the growth on the juice from these various sources. The growth in all cases was slight, slow in developing, entirely submerged, with few conidia, and these abnormal. When the potato extract from leaf or tuber was sterilized in the steam cooker, the growth obtained was about the same at the outset, but ultimately became somewhat more than that on the raw juice.

Broths from the potato and from various other vegetables were also tested in combination with gelatin or agar. This series included the following: Potato-broth gelatin, potato-broth agar, pumpkin-broth gelatin, pumpkin-broth agar, Lima-bean agar, prune-juice agar, starch gelatin, mannite gelatin, and various percentages of gelatin in pure water.

The potato gelatin was made up as follows: 1,000 grams of Green Mountain potatoes, after being washed, were cut into small blocks in 1,500 cubic centimeters of water. After cooking in the steamer for 1½ hours they were steamed in the autoclave at 2 pounds pressure for 1 hour. The liquid was separated by filtration through filter paper

and Nelson's gelatin added. Trials of various strengths showed that 10 per cent of gelatin gave the best results, although 7½ and 5 per cent gave results nearly as good. The gelatin was then dissolved by heating the medium in the steamer, after which it was cleared with white of egg, passed through filter paper by suction, and sterilized in the autoclave at not to exceed 4 pounds pressure for 15 minutes. A higher pressure or longer time prevented the gelatin from solidifying. Submerged inoculations were made in the earlier trials, either immediately under the surface or at the bottom of the tube. The gelatin was melted to permit the bottom inoculations. The growth in the potato gelatin medium, so handled, was fairly good and the hyphæ were normal in appearance. The production of the conidia was largely suppressed, whether in submerged cultures or in inoculations near the surface. In their stead there appeared the resting-spore development, which will be described in detail on a subsequent page. It was found that slant cultures could be made with this medium by increasing the gelatin to 12 per cent and that, when surface inoculations were made upon these, fairly abundant aerial growth developed. This result is in contrast to the experience of Clinton (1908, p. 899) with potato gelatin, he having found that it was a very poor medium, especially for aerial growth. Minor differences in medium or manipulation may account for this.

Potato-broth agar produced a slow growth which was ultimately fair but with fewer conidia than were produced on potato gelatin. Lima-bean agar has proved much better. The Lima-bean agar which has given us the best results is a modification of that of Clinton (1908, p. 898) and prepared as follows: 50 grams of Lima beans, after being ground as fine as possible in a meat chopper, were soaked for one-half hour in 500 cubic centimeters of tepid water, allowed to simmer for half an hour in the steamer, and the juice then strained through a cloth. After restoring the volume of broth to 500 cubic centimeters, 1 per cent of agar was dissolved in it by heating in the autoclave. Titration has shown this medium to have a reaction of about +4 Fuller's scale. The fungus forms on this medium in four to five days a good aerial growth of mycelium with many normal conidia. The resting spores, as later described, have been found on this medium, though less commonly than in the potato gelatin. This medium has proved to be especially useful for carrying the stock cultures of the different strains, since it is easier to prepare than raw-potato blocks and the fungus does not so soon suffer from desiccation. Therefore it has been exclusively used for this purpose during the latter part of the work.

On the pumpkin-broth agar and gelatin the growth while slow was ultimately fair. The development on these media has been chiefly

aerial with some conidia, but in much smaller number than on the potato-gelatin slants and the Lima-bean agar. On prune-juice agar there was no growth, showing that Phytophthora is unable to avail itself of the rich store of sugar of the prune. It is interesting to note that a small growth was secured in tubes containing simply gelatin (10 per cent) in water, this growth being estimated at two-fifths of that on potato gelatin. The addition of 0.5 of 1 per cent of starch to this gelatin had no material influence on the growth nor did the addition of 0.5 of 1 per cent of mannite.

GROWTH ON LIQUID SYNTHETIC MEDIA.

In addition to attempting to grow the fungus on some of the standard synthetic solutions, such as those of Uschinsky and Knopp, a further attempt was made to put together chemicals representing approximately the compounds found in the potato tuber. In this work the potato analyses of Watson (1895, p. 194) were used as a basis. He analyzed the ash of eight varieties of potatoes, Early Essex, Beauty of Hebron, Burbank, Green Mountain, Charles Downing, Early Rose, Early Ohio, and Sunrise, and gives the average of each of the constituents for these varieties to be as shown in Table VI.

TABLE VI.—*Average constituents of eight varieties of potato tubers.*

Constituents.	Average.	Constituents.	Average.
	Per cent.		*Per cent.*
Moisture	76.94	Magnesium (MgO)	0.054
Ash	1.35	Sulphuric acid (SO_3)	.076
Nitrogen	.394	Soda (Na_2O)	.063
Phosphoric acid (P_2O_5)	.154	Chlorin	.043
Potash (K_2O)	.828	Insoluble matter	.014
Lime (CaO)	.010		

In making up our liquid media it was practicable only to approximate the above composition percentages. Each of the combinations used contained some carbon compound and, if no nitrogen was supplied in the inorganic salts, some nitrogenous organic compound was added, such as asparagin. The growth that was obtained on these broths contributed nothing positive toward the main problems in mind, but they have interest as showing that a fairly normal growth of this fungus may be had on purely synthetic media and they contribute some facts of general interest concerning the nutrition of fungi. The composition of these various media and the resulting growth are shown in Table VII.

TABLE VII.—*Synthetic broths used for testing the growth of Phytophthora infestans.*

No. of broth.	Constituents added to 1,000 cubic centimeters of water.													Growth.
	Potassium phosphate K_2HPO_4.	Ammonium phosphate $(NH_4)_2PO_4$.	Potassium chlorid KCl.	Magnesium sulphate $MgSO_4$.	Calcium carbonate $CaCO_3$.	Potassium nitrate KNO_3.	Calcium nitrate $Ca(NO_3)_2$.	Calcium chlorid $CaCl_2$.	Glycerin.	Mannite.	Asparagin.	Potato starch.	Urea.	
I	1.00	10.00	.....	0.10	.....	.....	.....	.....	5.00	.....	.....	.....	.....	None.
II	1.00	10.00	.....	.10	.....	.....	.....	.....	.....	20.00	.....	.....	.....	Do.
III	1.00	5.00	.....	.10	.....	.....	1.00	.....	.....	20.00	.....	.....	.....	Do.
IV	.25	.....	0.05	.10	0.025	0.50	.....	.....	.....	.....	0.50	.....	.....	Good.[1]
V	.25	.....	.05	.10	.025	1.00	.....	.....	.....	.....	.....	.....	.....	None.
VI	.25	.....	.05	.10	.025	.....	.....	.....	.....	.....	1.00	.....	.....	Do.
VII	.25	.....	.05	.10	.025	1.00	.....	.....	.....	.....	.....	0.25	.....	Do.
VIII	.25	.....	.50	.10	.025	.50	.....	.....	.....	.....	.....	.....	0.50	Do.
IX	.25	.....	.50	.10	.025	.50	.....	.....	.....	.....	.....	.....	1.00	Do.
X	.25	.....	.05	.10	.025	.50	.....	.....	.....	.50	.....	.....	.....	Slight.
XI	.25	.....	.05	.10	.025	.....	.....	.....	.....	.50	.50	.....	.....	Good.[2]
XII	.25	.....	.05	.10	.025	.50	.....	.....	.....	.50	.....	.....	.50	Slight.
XIII	.25	.....	.05	.10	.025	.....	.....	.....	.....	.50	.....	.....	.50	Very slight.
XIV	.25	.....	.05	.10	.025	.50	.....	.....	.....	.50	.50	.....	.....	Fair.
XV	.25	.....	.....	.10	.025	.50	.....	.....	.....	.....	.50	.....	.....	Do.
XVI	.25	.....	.05	.10	.....	.50	.....	.....	.....	.....	.50	.....	.....	Do.[3]
XVII	.25	.....	.....	.10	.....	.50	.....	.....	.....	.....	.50	.....	.....	([4]).
XVIII	.25	.....	.....	.10	.....	.50	.....	0.08	.....	.....	.50	.....	.....	([5]).
XIX	.25	.....	.....	.10	.....	.50	.08	.....	.....	.....	.50	.....	.....	Slight.
XX	.25	.....	.....	.10	.....	.....	.50	.....	.....	.....	.50	.....	.....	None.
XXI	.25	.....	.....	.10	.....	.....	.50	.08	.....	.....	.50	.....	.....	Slight.
XXII	.25	.....	.50	.10	.....	.....	.50	.....	.....	.....	.50	.....	.....	Do.
XXIII	.75	.....	.15	.30	.075	1.50	.....	.....	.....	.....	1.50	.....	.....	([6]).
XXIV	2.50	.....	.50	1.00	.25	5.00	.....	.....	.....	.....	5.00	.....	.....	None.[7]
XXV	.25	.....	.05	.10	.025	.50	.....	.....	.....	.....	.50	.....	.....	Same as IV.[8]
XXVI	.025	.....	.005	.01	.0025	.05	.....	.....	.....	.....	.05	.....	.....	Fair.[9]

[1] Best liquid medium tried.
[2] About like that on medium IV.
[3] Hardly as good as that on IV.
[4] Good growth in two cases; in others varied from slight to fair.
[5] Growth varied in the different tubes from fair to good.
[6] This medium is IV×3. Fair growth in a few; in some cases very slight; very few conidia.
[7] This medium is IV×10.
[8] This medium is the same as IV except that untested "C. P." chemicals were used without verification.
[9] This medium is IV÷10.

The following comments will help in the interpretation of the table. It is noteworthy that the best growth was in No. IV, which is as nearly a duplicate of the analysis of potato ash as was found practicable with chemicals of known composition. It was found that a dilution of this medium to one-tenth of its original strength produced practically as much growth as the original medium. On the other hand, increasing the strength of the solution by using three times the quantity of chemicals still gave a fair growth, but with the broth that was made with ten times the quantity of chemicals no growth resulted. From this it seems that *Phytophthora infestans* can find a good food supply even in a very dilute solution, where the osmotic pressure is comparatively low, and that it is unable to grow in a solution with a relatively high osmotic pressure. This result is, of course, to be expected from the chemical composition of its natural

substratum, the potato, where the osmotic pressure produced by the inorganic salts is very low, and it is also in accord with the observation of Hecke (1898), already cited.

It may safely be assumed that one of the reasons for failure in Nos. I, II, and III is that the chemicals used were sufficient to produce osmotic pressure too high for the growth of the fungus. They contained 16.1 to 31.1 grams of soluble material as against 14.25 grams in No. XXIV, in which the concentration of the solution is clearly the cause of failure, as may be seen by comparison with No. IV. The absence of a suitable supply of nitrogen suggests itself as another possible explanation. In No. I nitrogen is supplied as ammonium phosphate and also as an impurity of the glycerin. In No. II ammonium phosphate is the only source of nitrogen, while in No. III calcium nitrate is added. The presence of glycerin may have inhibited growth in No. I, and the results with No. XIX suggest that calcium nitrate may have been a disadvantage in No. III. It may be noted that No. I is a modified Fermi solution.

When compared with No. IV, the results from No. V show that in the combinations employed asparagin is essential for growth, and the results from No. VI show that potassium nitrate is needed, or that an excess of asparagin inhibits growth. No. VII would seem to show that starch can not be utilized in place of asparagin, and Nos. VIII and IX would show the same concerning urea. In No. X it appears that mannite can replace asparagin to a very slight extent. It is hard to understand just why growth took place in No. XI and not in No. VI, but apparently the combination of mannite and asparagin sufficed to fill the lack of potassium nitrate.

From the results on these broths and the others not specifically mentioned it would seem as though it can be safely assumed that mannite, potato starch, urea, and calcium nitrate are not of appreciable use as a source of food for *Phytophthora infestans*. It would also appear that asparagin, magnesium sulphate, acid potassium phosphate, and possibly potassium chlorid are essential to its growth.

The remaining broths, Nos. XIV to XXII, all contained asparagin and on all of them more or less growth appeared, the amount varying with the other chemicals in the solution. The best of these are Nos. XIV to XVIII, inclusive. In No. XIX the addition of the calcium nitrate seems to have an inhibiting effect on the fungus and the growth is slight; at any rate the growth is not equal to that made in Nos. XIV and XV with calcium carbonate. The entire absence of calcium in No. XVII did not seem to prevent a good growth. This may be interpreted as a confirmation of the work of A. Mayer[1] on

[1] Mayer, A. E. Untersuchungen über die Alkoholischegährung, den Staffbedarf und den Stoffwechsel der Hefepflahze. Heidelberg, 1868, p. 44.

yeast, or Raulin[1] on Aspergillus, and Winogradsky[2] on *Mycoderma vini*, none of whom found calcium necessary for fungous growth. Nos. XX and XXI show, probably as a result of the lack of potassium, a very slight growth, while No. XXII with the potassium supplied as potassium chlorid seems to have had its growth inhibited by the addition of the calcium nitrate, as does No. XIX.

While the results with these broths are not complete, they indicate that *Phytophthora infestans* is limited to certain combinations of chemicals as sources of carbon, nitrogen, and energy. The only really efficient single carrier of these which was found is asparagin, and the availability of this substance seems to be dependent upon the presence of other chemicals, as may be seen by a comparison of Nos. IV, VI, and XI. In No. IV asparagin is the only source of carbon and energy; in No. XI it is the only source of nitrogen; in No. VI it is the only source of any of the three. It will be noted that good growth appeared in No. IV and in No. XI, but no growth in No. VI. Neither mannite nor potato starch could take the place of asparagin, even when combined with nitrogen in the form of potassium nitrate, and urea was not more suitable even when combined with mannite, as in No. XIII. The results also seem to show that certain inorganic salts such as calcium nitrate will have an inhibiting effect on the growth even when all the other food constituents are supplied, as in XXII.

GROWTH ON SYNTHETIC MEDIA WITH THE ADDITION OF AGAR OR GELATIN.

In connection with these trials a number of the standard liquid solutions for the cultivation of fungi were solidified with agar or gelatin and Phytophthora was tried on them. These included the Fermi, Uschinsky, and Knopp solutions and a number of modifications of a solution given by Heinemann (1907) as a potato substitute culture medium.

The regular Fermi gelatin was made by the addition of 10 grams of Nelson's photographic gelatin to 100 c. c. of Fermi's solution, the solution then being cleared by the white of egg. A modification was made in a similar way, except that it contained only 10 c. c. of Fermi's solution, to which 90 c. c. of water and 10 grams of gelatin were added.

The Uschinsky gelatin was made in the same way, using 100 c. c. of Uschinsky solution and 10 grams of gelatin.

[1] Raulin, J. Études Chemiques sur la Végétation. Annales des Sciences Naturelles, Botanique, ser. 5, vol. 11, 1869, p. 224.

[2] Winogradsky, S. Ueber die Wirkung äusserer Einflüsse auf die Entwicklung von Mycoderma Vini. (Arbeiten der St. Petersburger Naturforschende Gesellschaft, vol. 14, pt. 2, 1884, pp. 132–135.) (Russian.) Abstract in Botanisches Centralblatt, vol. 20, 1884, p. 167.

The Knopp gelatin consisted of 10 grams of gelatin similarly combined with the following solution: Calcium nitrate, 0.068 grams; potassium nitrate, 0.017 grams; magnesium sulphate, 0.017 grams; potassium phosphate, 0.017 grams; water, 100 c. c.

Heinemann's medium, as given by him (1907), contained the following: Magnesium sulphate, 2 grams; calcium chlorid, 2 grams; ammonium lactate, 2 grams; peptone, 10 grams; agar, 10 grams; starch, 10 grams; water (to make a total of 1,000 grams), 944 c. c.

In our procedure the same constituents were used, except for the omission of the peptone and the addition of potassium and sodium phosphate, but they were made up in three parts so that the proportion of agar, chemicals, and starch could be varied, as follows:

(1) Ten grams of agar in 400 c. c. of distilled water.

(2) Two grams each of dipotassium hydrogen phosphate, disodium hydrogen phosphate, magnesium sulphate, calcium chlorid, ammonium lactate; water, 200 c. c.

(3) Thirty grams of starch in 200 c. c. of water.

Five different mixtures of these three parts were used:

(A) 120 c. c. of 1; 40 c. c. of 2; 40 c. c. of distilled water.
(B) 120 c. c. of 1; 40 c. c. of 2; 40 c. c. of 3.
(C) 60 c. c. of 1; 40 c. c. of 2; 80 c. c. of distilled water.
(D) 80 c. c. of 1; 40 c. c. of 2; 40 c. c. of 3; 40 c. c. of distilled water.
(E) 40 c. c. of 1; 40 c. c. of 2; 40 c. c. of 3; 80 c. c. of distilled water.

Heinemann's medium, as he made it, is 5 per cent acid, but these modifications were all titrated and made neutral to phenolphthalein by the addition of sodium hydroxid.

The growth was only slight on the Fermi, Uschinsky, and Knopp gelatins and no production of conidia occurred. On the modifications of Heinemann's substitutes for potato a fair growth appeared after three days in the tubes of three of these variations (A, C, and E); a somewhat smaller growth appeared in another (B), and a slight growth in the fifth (D). A second titration of these media showed this last (D) to be slightly more acid than the others, and the difference noted was attributed to this fact. The other fact brought out was that the fungus thrived better in a medium without starch. The starch, even though in small amount, may present here the same mechanical obstacles to the advance of the fungus that it does in the cooked potato. The mycelial threads were found in these synthetic media to contain an abnormal amount of fat globules and to have penetrated the substratum to a considerable extent. In some cultures, where there was almost no visible growth, the medium was melted and the agar and starch, which rendered the medium semiopaque, were washed away, whereupon an abundant mycelial growth was revealed. Lateral branches of this mycelium, which were richly supplied with protoplasm, often showed 3 to 6 constrictions at intervals of 20 to 30

microns, which apparently represented abortive attempts at the formation of the resting spores noted in other cultures. The presence of an unusual amount of fat in the hyphæ is an indication that the fungus was not in normal condition and that none of these substrata are satisfactory substitutes for potato.

GROWTH ON SYNTHETIC MEDIA WITH SILICATE JELLY.

In order to compare the growth on solid and liquid media where the nutritive elements are the same, recourse was had to the use of silicate jelly. The synthetic broths Nos. I to IV, the constituents of which are shown in Table VII, were used, the chemicals being dissolved in only one-tenth the usual amount of water, and 10 c. c. of each was added to 90 c. c. of the silicate-jelly mixture. Growth appeared on all of these solidified synthetic media, the best being on No. IV. These growths did not, however, differ appreciably from those obtained on the corresponding liquid media either in quantity or character. This indicates that the fungus is indifferent as to whether its food occurs in a liquid or solid substratum and that a gelatinous material of the consistency of silicate jelly offers no serious obstacle to its progress.

BIOCHEMICAL AND THERMAL RELATIONS OF PHYTOPHTHORA INFESTANS.

In connection with the cultural studies already described, some observations were also made on the relations of varying degrees of acidity and alkalinity of the medium to the vigor and character of growth, as well as upon the temperature relations. These observations, while not complete in all respects, give some interesting evidence as to the resistance of the fungus to extremes of temperature and also as to its sensitiveness to the reaction of the substratum.

RELATIONS TO ACIDS AND ALKALIS.

The growths obtained on the various media used in the preceding studies, of which the reactions ranged from +14 to 0 Fuller's scale, have served to indicate that the fungus will survive a fairly wide range, being scarcely more sensitive to acids and alkalis than most of the bacterial plant pathogenes. In order to make more critical demonstrations upon this point, a special series of cultures was run on Lima-bean agar that had been made neutral to phenolphthalein and then reenforced with definite amounts of acid and alkali. Malic acid was chosen as the acid to use, since Snyder (1896, p. 85) shows it to be the one normally present in the potato tuber. Sodium hydroxid was used as the alkali. It was found that the carbon dioxid of the laboratory atmosphere seriously altered the reaction in such

alkaline culture tubes before the end of the five to seven days which the fungus required for characteristic growth. To avoid this difficulty, the inoculated alkaline tubes after inoculation were promptly placed in sealed glass jars, together with a tube of concentrated potassium hydroxid solution, and they were retitrated at the end of the experiment. Table VIII gives the results, the titrations being expressed according to Fuller's scale.

TABLE VIII.—*Relative rate of growth of Phytophthora infestans in acid and alkaline media.*

Titration at beginning of experiment.	Retitration at end of experiment.	Growth during experiment.
+40	+37.5	No growth.
+30	+25	Very little growth.
+20	+15	Some growth; no fruiting.
+10	+10	Fair growth; fruiting much less than on neutral medium.
0	+ 1	Growth and fruiting abundant.
− 5	− 3	Fair growth, but much less than on neutral medium; no fruiting.
−10	− 4	Fair growth in 2 tubes, slight in the other 6; no fruiting.
−15	− 8	Very slight growth.
−20	−12	No growth apparent.

From these results it will be seen that this fungus is more susceptible to the alkali than to the acid. The first effect of acid and alkali alike was to prevent the formation of the fruiting branches; but while the smallest amount, viz, −3, of the alkali did this, it required +15 acid to produce the same result. In contrast with this result was the wide range over which some vegetative growth may take place, viz, at least −8 to +25. The acid condition of the potato tissues would lead one to expect, of course, that the preponderance would be toward the acid end of the scale, but the inhibiting effect of even the smallest per cent of sodium hydroxid was so marked as to suggest that the lime present in Bordeaux mixture may have more fungicidal value than is generally supposed.

Acknowledgments are due to Dr. Mel T. Cook, of the Delaware Agricultural Experiment Station, for determining the effect of tannic acid upon the development of Phytophthora and for placing the results of his investigations at our disposal. He grew cultures of the fungus, sent for this purpose from our laboratory, on Lima-bean agar made according to the formula already given. To this medium tannin was added in varying proportions from 0 to 0.6 per cent, as follows: 0, 0.0067, 0.007, 0.0083, 0.01, 0.0125, 0.0167, 0.025, 0.05, 0.1, 0.2, 0.4, and 0.6 per cent. The experiment was repeated several times. Some variation was shown in the results, but the growth was better in all media without tannin than where even the smallest amount of tannin was present. Tannin of the five lesser strengths, 0.0067 to 0.0125 per cent, gave a somewhat smaller growth, but it still was fairly luxuriant and practically the same throughout. From 0.0167 to 0.05

per cent the growth was appreciably less than in the weaker series but without much difference within these limits. From this point there was a marked decline in vigor and from 0.2 to 0.6 per cent, inclusive, there was practically no growth at all. Dr. Cook's primary aim in his studies, which have included many other fungi (1910, pp. 751–752), is to learn whether tannin may be a factor in determining relative disease resistance. While this may be the case with certain hosts and parasites it seems hardly probable with the potato, since its normal tannin content is very low.

THERMAL RELATIONS.

Considerable work has been done by previous investigators on the temperature limits for the life and growth of *Phytophthora infestans*, and these investigations supplemented by the following studies show that its growth is favored by a relatively low temperature while the fungus survives a lower temperature at least than the host plant. Jensen (1887) concluded from his trials that a temperature of 26° C. was fatal to the mycelium in the tubers, while only 25° C. was necessary to kill the spores. Jensen's conclusions as to the probable relation of this thermal death point to the delay in the transfer of the fungus from South America to Europe were reviewed earlier in this bulletin, as was also his ingenious method of tuber disinfection by dry heat. More recently Matruchot and Molliard (1903, p. 524) report that growth in culture stops at 30° C., but they did not determine the thermal death point.

Our own determinations of the thermal death point of the fungus lead us to place it somewhat higher than Jensen did; but the methods used are not strictly comparable, since our trials were made with cultures in potato gelatin. Four strains were tested, one tube of each strain being subjected to each temperature. After inoculation the gelatin in these tubes was carefully warmed to the melting point, control tubes being similarly melted at the same time. The experimental tubes were then immersed in a water bath the temperature of which could be controlled to one-tenth of a degree centigrade. The tubes were immersed four-fifths of their length and a thermometer was placed in a control tube beside them. The tubes were exposed for 10 minutes after the temperature inside the control tube had reached the desired point.

Exposures were made at temperatures ranging from 29° to 50° C. No inhibiting effects resulted from such heating at temperatures below 37° C., but some of the tubes carried above this temperature failed to grow. The lack of uniformity in these results was attributed to the fact that the culture tubes used in these trials were thick walled and not very uniform. A second trial was therefore made,

using very thin-walled tubes. Good growths appeared in all tubes exposed to temperatures up to 40° C., but beyond this the results as to growth were doubtful.

The ability of the fungus to withstand cold is important in view of its life history in its native habitat, where it must pass the period of dormancy in some way in the soil at relatively low temperatures. Matruchot and Molliard (1903, p. 542) found that the growth was rapid at the average temperature of their laboratory (about 15° C.). The best temperature for good growth in tube cultures on raw-potato cylinders or other media was found in our trials to be about 16° to 18° C. The growth of the fungus at low temperatures was tested by the following experiment: Forty tubes of Lima-bean agar after inoculation with the Phytophthora were placed in four compartments of a low-temperature incubator, 10 tubes being placed at each temperature. Examinations and comparisons were made at intervals up to 22 days. A similar experiment was conducted with high temperatures. The results are shown in Table IX.

TABLE IX.—*Experiments showing growth of Phytophthora at different temperatures on Lima-bean agar.*

Experiments at low temperatures.			Experiments at high temperatures.		
Compartment.	Temperature.	Growth.	Compartment.	Temperature.	Growth.
	° C.			° C.	
1.....	3 to 5	None perceptible.	1.....	30	None apparent.
2.....	10 to 11	Fair after 22 days, but slow in appearing.	2.....	27	Fair; no fruiting.
			3.....	25	Do.
3.....	16 to 18	Abundant; much fruiting.	4.....	23	Good; a little fruiting.
4.....	18 to 19	Do.	5.....	21	Good; abundant fruiting.

Some attempts were made to determine the low thermal death point of Phytophthora in potato-gelatin cultures. The results indicate that the fungus in such cultures will withstand freezing temperatures fatal to the potato tuber. Since, however, only one series of trials was made it is deemed best to withhold definite conclusions until this work can be repeated.

PRODUCTION OF RESTING SPORES BY PHYTOPHTHORA INFESTANS.[1]

The members of the group to which this potato fungus belongs typically produce sexual spores, oospores, which function as resting spores. One of the keenest controversies in mycological history has concerned the question as to whether *Phytophthora infestans* pro-

[1] This matter was prepared for publication in 1910. Just before sending it to press Clinton's interesting announcement was received concerning the discovery of oospores of the potato fungus. It has not seemed wise to attempt any revision of this text, but instead some statements are added in a footnote at the close of this chapter, page 69.

duces such resting spores or whether, on the contrary, it is wholly dependent for overwintering on the mycelium infesting potato tubers. De Bary in his early studies had this question in mind, but his researches (1876) failed to discover any form of resting spore. W. G. Smith (1875, 1876) later found bodies which he said were such oospores, and Vize, Broome, Plowright (Smith, 1876), and other English botanists accepted his evidence. On the other hand De Bary disputed Smith's claims, and other mycologists, Berkeley (Smith, 1876), Cornu (1881), and others, were drawn into the discussion. The outcome has been the general acceptance by modern botanists of De Bary's verdict, "not proved," regarding Smith's work. With the exception of a single paper by Smorawski (1890) no one has reopened the question during the last 25 years.

As already stated, attention was turned to the development of methods of handling *Phytophthora infestans* in pure culture in the hope of gaining further light upon the question. While the results are not by any means conclusive they at least give encouragement for further effort. Before outlining them, a summarized account of the observations and conclusions of Smith, De Bary, and Smorawski is needed for purposes of comparison.

EARLIER INVESTIGATIONS.

In 1875 W. G. Smith published his first announcement of the discovery of what he claimed were oospores of *Phytophthora infestans*. He obtained these spores by placing potato leaves infested with this fungus in a moist chamber and allowing them to decay. Upon later examination of this decaying material he found oogonia and antheridia and oospores with walls either smooth or spiny and he concluded that these belonged to the potato fungus. The inconclusiveness of this evidence is apparent, since decaying leaves are open to the invasion of various saprophytes. The following year Smith announced (1876) that he had been able to germinate these overwintered resting spores and from them came a germ tube on which was produced almost immediately a sporangium like that typical of *Phytophthora infestans*. These results were accepted by Vize, Broome, and Plowright (Smith, 1876), who had followed his studies.

In the meantime De Bary, under the patronage of the Royal Agricultural Society, had been employed in studies upon this same fungus, and in the journal of that society for 1876 (De Bary, 1876) he published the results which led him to discredit Smith's conclusions. Examining potato leaves, as Smith had done, which were in process of decay following the attacks of *Phytophthora infestans*, he found numerous fungus developments within them, two of which resembled Smith's so-called oospores. One of these he showed to be a saprophytic Pythium, which, because of the trouble it had given, he called

Pythium vexans; the other he identified with the *Artotrogus hydnosporus*, which Montagne had described in 1845. These spores were borne either at the tips of branches or intercalary on hyphæ showing some septation near the spore. In the tissues of potatoes penetrated by the mycelium of Phytophthora he found other bodies which suggested oogonia or oospores of the potato fungus. He interpreted these also as *Artotrogus hydnosporus*, basing his conclusion largely upon the brief description and figure published by Berkeley in 1846. Montagne described two kinds of these spores, a smooth-walled and a spiny one, but named the fungus from the spiny one. Montagne evidently regarded the two as stages of the same species, the smooth-walled one being the less mature, but De Bary was inclined to believe that while they were locally associated they were distinct. No antheridia were discovered, even after the most diligent search, and the spores were evidently to be regarded as resting spores of some unknown fungus. With regard to the relation of *Artotrogus hydnosporus* to *Phytophthora infestans*, De Bary (1876, pp. 256–257) has this to say:

> In most cases I found these bodies complete, mature, and without any distinct indication of their being attached to mycelium. It was certainly remarkable that they were often situated close to the inner surface of the cell walls in places where externally the mycelium of Phytophthora undoubtedly ran in the intercellular spaces, or even where a short branch of it penetrated the interior of the cell.

All these phenomena were reconcilable by the conjecture that the prickly bodies might perhaps be the long-sought oospores of Phytophthora; but, on the other hand, they might be of quite different origin and their bearers or producers have disappeared. A little farther along (p. 257) he says:

> There is no reason whatever to consider them as belonging to the potato fungus, unless we base it on the fact that I found them close to that fungus in the course of experiments in search of its oospores.

De Bary says, however, as to their origin: "They grow on the extremities of the branches of a mycelium, which is very like that of *Pythium vexans*." From these quotations it will be seen that *Artotrogus hydnosporus* was a puzzle to De Bary, as it had been to Montagne and Berkeley, and that, while he evidently believed that it bore no relation to *Phytophthora infestans*, he was never able to establish their distinctness.

Cornu (1881, p. 104) examined the bodies found by Smith and also the *Artotrogus hydnosporus* of Montagne Smith's oospores, he seemed inclined to believe, belonged to some species of Pythium. His studies included chemical tests on the walls of the Artotrogus spores. He concluded that nothing in the nature of these spiny-walled spores is opposed to the idea that they belong to *Phytoph-*

thora infestans, or if not to that, then to a Saprolegnia different from any with which he was familiar. He is careful, however, not to commit himself fully to the idea that they actually do belong to the Phytophthora.

Smorawski (1890) used the same methods as De Bary and Smith, simply teasing apart some of the diseased potato leaf or tuber in water. It was in a tuber that he was able to see what he interpreted as the sexual organs of the fungus. On the side branch of a conidiophore he observed two short thick branches; one of these was sack shaped and the other approached it and apparently fused with it. The only differentiation in the oospore, however, was a dark fat-containing mass at the center surrounded by a peripheral, extremely fine-grained layer of periplasm. He admits that it was impossible at any time to see the actual pressing in of the antheridial tube into the oogone. For this reason he does not deny the possibility of a parthogenetic development of the oospores.

Here, then, is the present state of the problem. All students of Phytophthora, reasoning from the behavior of the related Peronosporales, admit the possibility, if not the probability, of the occurrence of resting spores. Moreover, it is difficult to account for the occurrence and distribution of the fungus simply by means of mycelium overwintering in the tubers. Nevertheless, the claims of Smith and Smorawski, both of whom report the observance of oospores, have failed to carry conviction because of lack of accuracy in the methods followed. The most critical students have, however, admitted the possibility, remote though it be, that Montagne's *Artotrogus hydnosporus* may be the resting spore of Phytophthora.

STUDIES OF THE PROBLEM OF RESTING SPORES AT THE VERMONT STATION.

We will now review our own investigations bearing upon this question. During the winter of 1904–05 W. M. Gambell, working as a student in our laboratory with pure cultures of *Phytophthora infestans* on raw potato, observed occasional thick-walled sporelike bodies. All told he found only eight such, but since they occurred in different cultures and were quite similar they seemed significant as possibly immature resting spores. They were lying embedded in the mycelium, close to the surface, but external to the potato blocks, 22 to 23 microns in diameter, spherical, with a single smooth wall, 2¼ to 3 microns thick, which was in some nearly colorless and in others browned, the contents coarsely granular. In most cases their attachment to the mycelium could not be seen clearly, but they appeared to arise terminally on branches modified but little if any. A few more bodies essentially like these have since been found on our raw-potato cultures, but in no case have they appeared to us to be normally matured functional resting spores, although they were

clearly of some unusual nature, resembling resting spores rather than the ordinary conidia of Phytophthora. Cultures were carried continuously during the next three years, at first on raw potato and potato extracts and then on the various other forms of culture media as already described. Although microscopic examinations were made of the growths on these media, nothing of further interest was seen until some potato-gelatin cultures were under examination in 1908. The growth in this gelatin medium is chiefly submerged, with a meager development of aerial branches and sporangia. Occasional brown specks, barely visible to the naked eye, were detected in the submerged mycelium. Under the microscope these specks proved to be clusters of thick-walled sporelike bodies essentially like those previously found in the raw-potato cultures, which became thus visible to the naked eye because of a brown staining of the potato gelatin in their vicinity. Particular attention was given thereafter to the growth of the fungus in this medium and, thanks to the development of the brown stain in their vicinity, these bodies, although never in great abundance, have been found frequently enough to permit careful study.

In order to prepare the mounts for microscopic examination it was necessary to melt the medium. The mycelium could then be lifted out en masse. By heating the medium in a 5 per cent solution of potassium hydroxid, the bulk of the gelatin adhering to it was removed and the material was then easily examined in glycerin and permanent mounts made in glycerin jelly. In some cases the transfer was made directly from the melted culture medium to glycerin and thence to glycerin jelly in order to avoid possible alterations by the caustic solution. The growth of the fungus in potato gelatin is never very rapid or abundant. The development of these bodies does not begin until the vegetative growth has passed its greatest vigor, which at a temperature of 20° C. has usually been some time during the second week. The brown stain is slight at first but deepens with age. Much variation in structure, grouping, and mode of development of these bodies has been observed, partly due to variations in medium. Most of these bodies have clearly been abnormal developments, or at least have failed to reach normal maturity. Indeed, we doubt if any of them are to be regarded as strictly normal. Nevertheless, it seems worth while to figure and describe the more common or striking features observed. In some cases some of these bodies were developed intercalary in a hypha, which differed from the ordinary vegetative hyphæ merely by the local enlargement and thickening of the walls (Pl. V, figs. 18 and 22). Usually, however, they were terminal and single and the hypha bearing them was enlarged and thickened for some distance backward (Pls. IV and V, figs. 1–12). Frequently the branch forked, bearing two or more of the bodies terminally

(Pl. V, figs. 11 and 12); the forking was in some repeated several times in quite regular dichotomy with the separate branches fairly long (Pl. IV, fig. 10) and in others they were less regular and short so as to bring numerous bodies into a rather dense irregular cluster (Pl. IV, fig. 1). The mycelium in old cultures of Phytophthora shows frequent septation, and this usually occurred in the vicinity of those bodies, but there seemed no point where a septum regularly occurred to delimit the enlarged sporelike end from the ordinary vegetative hypha. The wall of the enlarging end immediately adjacent to the protoplasm was sharply defined, thickened, and soon became distinctly browned, the color increasing with age. In our first series of cultures on potato gelatin this wall was bounded externally by a much thicker, clear, refractive layer (Pl. IV–V, figs. 1–16), which commonly showed some of the brown coloration, but might remain clear. At the periphery this layer was in some specimens quite smooth, with faint but distinct delimitation (Pl. V, figs. 11 and 12), the granules being apparently in part the roughened surface of this layer itself and in part the granular matter of the medium. This layer itself was interpreted to be an abnormally gelatinized surface layer of the wall, probably comparable to the exospore of the more normally developed spores to be described later.

It is to be remembered that these bodies wherever found were developed on the submerged hyphæ in the gelatinous medium and that the characters of the wall both as to coloration and thickness were doubtless in some degree modified thereby. The protoplasm was denser and more coarsely granular in these sporelike enlargements, with large vacuoles, and frequently contained relatively large oil globules. There was no differentiation of central from peripheral portions comparable to oosphere and periplasm except in one case (Pl. V, fig. 11). This bore a striking resemblance to a young oospore, but since its occurrence was exceptional and the medium so abnormal but little importance can be attached thereto. Embedded in the gelatinous outer envelope there occurred in many cases in our earlier cultures small bodies the nature or function of which was not apparent. These were lens or saucer shaped, clear, highly refractive, and often with what appeared to be a vacuole. It may be recalled that Stevens (1899, fig. 91) found somewhat similar bodies in the early stage of the developing exospore of *Albugo bliti*, but without vacuoles. This vacuolelike appearance suggests a protoplasmic nature for the bodies, but that idea does not seem tenable. Since the bodies have not been seen in our more recent cultures they must be regarded as abnormalities or possibly even as artifacts. The discovery of these sporelike structures, even though evidently abnormal, stimulated to further cultures in the hope of obtaining normally matured spores. For these, both potato gelatin and Lima-bean agar have

been used. These further studies have added considerably to our knowledge of both the early stages and of the later developments of these bodies. In the very young cultures from the potato gelatin it was found that the production of typical conidia ceases soon after transfer to this medium. Instead, the bodies already described have generally soon appeared, their numbers reaching the maximum in six to eight days, and in certain cases they have developed at about the end of the second week into what appear to be mature resting spores with thick spiny walls. Even these later developments can not be accepted as entirely normal, but since they are evidently much more nearly so than the earlier ones the details will be reviewed as to their development and structure. Since no antheridia have been found and the nature of the bodies is uncertain we shall call them resting spores.

The swellings are commonly terminal, but may be intercalary; in which case they are more apt to arise at a point where the hyphæ branch. The hyphal walls immediately adjacent are swollen to several times the normal thickness and may show evidence of stratification. The walls in the early stages are also thickened and as a rule are externally smooth. A few have been observed, however, having external protuberances suggestive of the early stage of spine formation. The protoplasm extends into these protuberances so as to form a stellate mass in cross section (Pl. VI, figs. 25 and 26). At this stage the walls, which at first were colorless, were becoming light yellow and when sectioned ceased to take up analin stains, as they had done earlier. Where the protoplasmic protuberances extended, spines apparently were finally developed by the shrinking back of the material between. With approaching maturity the protoplasmic arms were withdrawn into the central mass, which thereupon became a sphere.

Similar stages were observed by De Bary in the development of the spores of *Artotrogus hydnosporus*. He records (1876, p. 257) that the external prominences of the wall were at first short and blunt, then developed into longer sharp spines, the protoplasm projecting at first into these and being later withdrawn into a globular mass inclosed in a smooth double membrane. The noteworthy difference is that our spores of this mature spiny-walled type show but a single cell membrane.

In early form and ultimate size these spores have shown considerable variation. If terminal, they were approximately spherical, but if intercalary at the forking of a hypha they may be quite irregular (Pl. VI, figs. 34, 36, 38). They are in contrast in this respect with the spherical form that is found in the loose mycelium growing on the raw potato. Doubtless this variation from the regular spherical form is simply due to the growth tension acting with the external

pressure of the embedding culture medium. In diameter they have varied from 20 to 50 microns. Those that have matured sufficiently to form spiny walls are, however, more uniform, the usual sizes ranging from 21 to 33 microns. A tendency has been noted toward two extremes in size, with fewer of the intermediate sizes. No tabulated record of measurements has been kept, but we estimate that about one-half of all mature spiny-walled spores we have seen have been of the larger type, i. e., 30 to 33 microns diameter, one-fourth of the smaller type, i. e., 21 to 22 microns, and one-fourth intermediate between these two. It is probable that this difference in size is merely due to nutrition. In some cases the two sizes were borne side by side and on the same thread (Pl. VI, fig. 36). In most cases the spiny-walled spores had become detached from the threads on which they were borne and were found lying free and singly. A few have been observed attached singly to short sporophores and a few have been found clinging together in clusters of two, three, or even a much larger number (Pl. VII, figs, 39–41).

A considerable variation has been observed also in the thickness of the wall and the character of the spines. Taking the larger type (Pl. VI, figs. 33, 34, 38) as the more normal, the measurements were in general: Diameter 31 to 33 microns; thickness of wall, 3 to 4 microns; length of spines borne upon the wall, 2.5 to 4 microns. The wall does not in all cases, and perhaps in none, develop uniformly throughout. Usually three or four thinner or weaker places occur which have spines less strongly developed (Pl. VI, figs. 30–38). Perhaps these were in some cases the points of attachment to the sporogenous hyphæ. The regularity of their distribution and the ease with which they might be ruptured at this point suggested that they might function as germ pores. In some cases a short hypha extending from one or more of these suggested premature germination or else a reversal to the vegetative type of development. No further growth of such tubes has been observed by us, however. It is worth noting that De Bary (1881, pl. 1, figs. 22–26) observed something similar in his *Artotrogus hydnosporus* and that W. G. Smith in his drawing shows some similar markings. They also bear some resemblance to the papilla developed by the oogonium of certain Peronosporales, e. g., *Albugo bliti*, for the reception of the antheridial tube. Of course, the question of germination of these spores has been kept in mind but nothing has been seen beyond what is described above. Until the spores are found in larger numbers and with such uniformity in character as to give assurance of normality there is, indeed, little to encourage any attempt to germinate them. The relative sparseness of these mature forms has also made microchemical studies difficult. It has been found that the walls in the young stages readily take up gentian violet, but that as soon as they begin to turn

yellow they refuse to stain. Various reagents have been tested on the mature spiny-walled spores without avail. They do not react to chlorzinc iodid, nor are they swollen or dissolved either by hot caustic potash (5 per cent solution) or by hot, strong hydrochloric or sulphuric acids. Nuclear stains were used with fair success upon the vegetative hyphæ and the early stages of these spore developments. Good stains were obtained alike whether treated in bulk or after embedding in paraffin and sectioning. Iron hæmatoxylin and Fleming's triple stain (safranin gentian violet and orange G.) were used. The vegetative hyphæ show the multinucleate condition typical of the Oomycetes. No attempt was made to differentiate the parts of the nucleus but many of them showed the nucleolus. The tendency to elongate parallel with the length of the hypha was apparent here as in the hyphæ from the potato leaf described on an earlier page (Pl. VIII, fig. 48). The sporelike bodies could be stained only in the earlier stages, i. e., until the walls began to brown.

The older stages were not very numerous and, while a few were found in the sections, these seemed to be filled with fat globules and none of them were so stained that anything could be made out as to nuclear conditions. In the younger stages, however, the bodies were multinucleate, having from 30 to 50 nuclei (Pl. VIII, figs. 42–46). These nuclei were even more elongated than those of the hyphæ, suggesting that they had been elongated with the inflowing of the protoplasm to form the body. In the later stages they were more rounded and nucleoli could be distinguished. In addition to nuclei there appeared in some of the older bodies, which were just beginning to show brown coloration of the wall, a body about four times the diameter of the nucleus. This body was fairly regular in outline and seemed filled with small dark-staining granules in some sections (Pl. VIII, figs. 42 and 46), while in others (Pl. VIII, fig. 47) it contained vacuoles. At all times, however, the staining was darker than the surrounding cytoplasm. This body may be comparable to the cœnocentrum noted by Wager (1896), Davis (1900), Stevens (1901), and others who have worked on the oospore development of this group. It allows, however, of another interpretation in view of the circumstances of its development. In size, shape, and contents it resembles the early stages of development of the oosphere in the various Peronosporales. It is true that the later stages of this body are not comparable to that, inasmuch as they then show unmistakable signs of disintegration. If we regard these bodies as oospores developed parthogenetically this central body may constitute stages in the development of the oogonium. The true nature and possible function of these bodies can at this time be nothing more than conjecture.

The preceding description has been based on the spore development obtained on potato gelatin, the medium in which they were found most abundantly. Similar bodies, including the brown spiny-walled mature spores, have been found in Lima-bean agar cultures, although only sparingly.

The most abundant development of these resting spores has been secured in submerged cultures in potato gelatin. The question has naturally arisen as to why this is so. On raw potato, where the vegetative development is most vigorous and the typical asexual spores, or conidia, are produced in greatest abundance, very few of these resting spores have been found. On Lima-bean agar there is moderate vegetative growth and sporangium production and some such resting spores, but fewer than on gelatin. Moreover, on the gelatin this type of spore formation does not occur until after the period of maximum vegetative vigor. It seems clear, therefore, that the suppression of vegetative vigor and of conidial formation is a first condition of the formation of these resting spores. The fact that they have been found only on the submerged growth in this gelatin medium further suggests that lack of oxygen may be a stimulus. Various experimental cultures were made to determine whether or not this was so. These have included attempts to stimulate their formation by keeping cultures on Lima-bean agar and potato gelatin in flasks from which the air has been partly or wholly exhausted, being replaced when wholly exhausted by carbon dioxid. No resting spores were obtained, however, by either treatment. Cultures in another series were started on the surface of either gelatin or agar tubes and then buried by pouring over them a thin layer of plain gelatin or agar solution. In most tubes no spores were developed, and where they were found in certain of the agar tubes it was not clear that the treatment was the direct cause. No further conclusions seemed justified therefore as to the relation of oxygen to their formation. It is to be recalled in this connection that wholly submerged growths have been secured on liquid potato extracts where very few conidia and no resting spores occurred.

The resemblance of these bodies to oogonia is evident, especially in the earlier stages of their development. Persistent search has, however, failed to discover any associated bodies resembling antheridia. Of course the possible relation of nutrition to antheridial development has been kept in mind. Klebs (1899, 1900) and Kauffman (1908, p. 377) have shown that the addition of certain elements to the culture medium may influence the development of antheridia in the case of Saprolegnia. As already explained, trials were made with a wide range of media but without positive results in this respect. The possibility that the development of such sexual organs might occur only when the growth of the fungus of two or more strains in-

termingled has been in mind from the outset of the work with these cultures, and Clinton (1905, pp. 326–328) has suggested this same idea. Two or more strains of the Phytophthora were grown intermingled upon raw-potato slices at various times in our earlier studies and a careful examination was made of the resulting mycelium, but no unusual developments occurred. After the discovery that potato gelatin was the most favorable medium for the development of these bodies more extensive trials were made with combination cultures in tubes of this medium. Nine strains of Phytophthora obtained, as already explained, from widely separated places in America and Europe were then in culture. These nine strains were paired in all the 36 possible combinations. In order to test the matter more thoroughly two cultures of each combination were started by inoculations made upon the surface of the medium, and two others where the inoculations were plunged beneath the surface so that the growths might be submerged from the outset. Thus each of the 36 pairs of strains was grown in four tubes, making a total of 144 cultures. Moreover, these cultures were repeated at four different times. Examinations of these mixed cultures revealed no unusual developments, either in kind or number of the bodies, as compared with the cultures of single strains, which were always made as controls. It is possible that further trials with other media or different environmental conditions might lead to other results. Under the circumstances, however, the only conclusion which seems justifiable is that there is no evidence of sexual differentiation of these Phytophthora strains and that these bodies are produced asexually.

Of course it is possible that resting spores of this sort, produced under the abnormal conditions of our culture tubes, may have no counterpart in nature. Brefeld (1873) and Klebs (1896) have both recorded evidence of the abnormalities which may result in fungus cultures under abnormal cultural conditions. Certainly no practical conclusions can be drawn from these observations, except as they may be supported by evidence obtained from more natural growths. Diligent and repeated searches upon pure cultures on raw potato in tubes, as previously explained, have led to the finding of but very few of these bodies, and these few were smooth walled, apparently immature, and of somewhat doubtful character. We have no encouragement, therefore, for the belief that Phytophthora forms resting spores with any degree of regularity or frequency in the tissues of the potato tuber.

Reference has already been made to the fact that Smith (1876) and others have found resting spores in decayed potato leaves following the invasion of Phytophthora, and these have been doubtfully regarded as belonging to this fungus. These observations have been

repeated in our laboratory by taking leaves which were thoroughly invaded by Phytophthora and placing them in a glass dish lined with moist filter paper. Of course such leaves are promptly rotted with the development of saprophytic bacteria and fungi. Examination after a couple of weeks has generally shown numerous resting spores (Pl. VI, fig. 28) corresponding in a general way with those described by others and somewhat similar to some of those found in our culture tubes. The origin of these spores has not as yet been traced to our satisfaction, nor have we been able to germinate them. In view of the historical evidence, and the abundant development observed of secondary saprophytes, the presumption must remain that these resting spores occurring in decaying potato leaves are of Pythium or some related saprophytic species. But the matter merits renewed attention.

The only evidence here presented which is of much importance is based upon the cultural work, the validity of which rests upon the purity of our cultures. A summary of the reasons for our confidence in their purity is therefore here given. These spores have been found in nine different strains of Phytophthora, which were all that were being carried in our laboratory at the time of these later studies. All but one of these nine strains were obtained from diseased potato tubers and isolated in our laboratory; the one exception was sent us by Dr. Clinton from Connecticut. These tubers were obtained from widely different sources including Vermont, Maine, Connecticut, Pennsylvania, England, Ireland, and Germany. The strains were isolated at different times and by three different persons. Some difficulty was usually experienced at the outset in eliminating bacteria from these cultures, but no trouble was experienced with fungi. These nine strains were carried continuously in culture for over three years without anything occurring to throw suspicion on their purity. Repeated transfers from these cultures to ordinary bacteriological media, nutrient broths, agar, and gelatin have uniformly failed to secure growth, as likewise have transfers to cooked potato and other vegetables. This fact seems to rule out the occurrence of any ordinary type of saprophyte. Inoculation experiments at various times have been made from these cultures to potato leaves upon greenhouse plants, and characteristic infections have always been promptly obtained showing that the Phytophthora persists with virulence unimpaired. It is not believed possible that any admixture of saprophytic growth could have entered all cultures alike, much less persist without detection. The only suggestion that seems worthy of further consideration is that these resting spores might belong to a species parasitic upon Phytophthora as Piptocephalis is upon certain molds. De Bary, indeed, suggests (1881) such relation as possible between *Artotrogus hydnosporus* and *Pythium debaryanum*. It would seem to us almost impossible, however, that such a condition should occur

in all nine cultures alike and persist without detection during so long a period and under such varied cultural conditions.[1]

DISEASE RESISTANCE OF POTATOES.

A historical review and summary of the main facts as to the disease resistance of potatoes were given by the senior writer in an earlier publication (Jones, 1905). As there pointed out, differences were observed in the liability of potato varieties to the ravages of blight and rot almost from the appearance of the disease in Europe. The necessity for systematic attempts for varietal improvement in this respect was clearly recognized in England over 30 years ago. The English Parliament was asked to give direct aid to this project in 1880. American varieties were sought as a basis for improvement of the English strains and hybridizing was practiced. The best production of the early English breeders was Magnum Bonum, the result of a cross of the American Early Rose with the English Victoria in 1876. The superior disease-resisting qualities of this cross not only made it the standard main-crop variety, but stimulated further attempts at the production of disease-resisting varieties. Indeed, disease resistance has since been a clearly recognized aim of all British potato specialists. Attention has more recently been turned toward the same goal in Germany and elsewhere on the continent of Europe. The senior writer, in 1904, as special agent of the Department of Agriculture, obtained a collection of the most promising European varieties for trial in America, together with a representative series of American varieties. These varieties have since been tried in the field at the Vermont experiment station by William Stuart, and elsewhere under the supervision of W. A. Orton, of the Department of Agriculture. Stuart's results, which have received preliminary consideration in a Vermont station bulletin (1906) and which later will be referred to more in detail, showed certain German, English-Scotch, and Dutch varieties to have a much higher degree of disease resistance than the standard American varieties.

While this field work has been in progress efforts in the laboratory studies have been directed along two lines: (1) To test, and if possible perfect, methods of determining relative disease resistance by inoculation with Phytophthora; (2) to learn any facts concerning the location and possible nature of the Phytophthora-resistant qualities.

[1] After the above matter was prepared for publication, Dr. G. P. Clinton's very interesting publications were received reporting the discovery of oospores of the potato fungus (Science, n. s., vol. 33, May 12, 1911, p. 744, and Report of the Connecticut Agricultural Experiment Station, 1909–10, distributed June, 1911, p. 753). Time has not permitted repetition in our laboratory of his cultural studies. The reading of his report is recommended, and also trial of the new medium to which he attributes the development of antheridia, viz, 50 grams ground oats (grain), 350 c. c. water (steamed), 10 grams agar (steamed), water to make 500 c. c. (autoclave 15 minutes at 7 to 10 pounds pressure).

The practical importance of both of these matters should be clear. All the previous trials to determine relative disease resistance have been based on field observations as to the occurrence of blight and rot following natural infection. The tardiness and uncertainty of this method are shown by Stuart's experience, since in his trials covering the six years 1905 to 1910 he has had only one year, 1905, when there was such a uniform and abundant development of the fungus upon his fields as is necessary to furnish reliable data. Moreover, even when the required development does occur, much allowance must be made for minor variations in atmospheric and soil conditions, and data so secured, especially as to rot, are not altogether satisfactory.

On the other hand, the potato specialist who is endeavoring by breeding or selection to develop more highly resistant varieties has urgent need for more information as to what constitutes disease resistance. Is it a matter of habit of growth, or epidermal texture, or other characters, or does it reside in the internal tissues and, if so, is it due to the presence of some recognizable chemical compound? With these questions in mind, inoculation studies were made on both potato leaves and tubers.

STUDIES OF PHYTOPHTHORA RESISTANCE AS SHOWN BY POTATO LEAVES.

Frequent trials have shown that leaf infections are easily obtained by applying Phytophthora spores to moistened potato leaves, provided moisture conditions are properly maintained. The main object in the present experiments with the foliage infection was to learn whether there would be a noticeable difference in either the percentage of infection or the rate of spread through the leaf tissues, following artificial infection of varieties reputed to be disease resistant, as compared with those considered more susceptible. Of course it was not practicable to carry on such a trial as this in the main variety field, since there would be too much danger from the proximity of so many susceptible varieties.

The German variety Irene, the English variety Holborn Abundance, and the American variety Ionia Seedling were selected as the most promising ones available in the smaller garden where the work was undertaken. Stuart's earlier experiments (1906, p. 110) had shown these varieties to have considerable difference in foliage resistance to Phytophthora. No late-blight was present on the plants when the work was undertaken, nor did it develop elsewhere in the field while the experiment was under way. Twenty leaflets occurring on three hills of each of these varieties were inoculated August 1 with Phytophthora. The weather being dry and warm, precautions were necessary to insure moisture conditions favorable for the development of the fungus. This need was successfully met by wet-

ting down the plants and adjacent soil thoroughly, making the inoculations in the late afternoon, and covering each plant with a barrel during the first night. Fresh spores of Phytophthora from potato leaves were brought from another field which was blighting as a result of earlier artificial inoculation. These spores were applied in a drop of water near the end of the leaflet under trial. The inoculated leaflets were tagged and numbered and the outline of each traced on thin semitransparent paper, to be used as a record sheet for following the progress of the disease. On August 5 the first sign of dying tissue was evident and its outline was traced with pencil in the corresponding leaf outline (fig. 8). Two days later, when the spots had increased, new tracings were added, and this process was

FIG. 8.—Diagrammatic representation of the rate of progress of *Phytophthora infestans* in two leaflets of the Ionia Seedling potato from the fourth to the eleventh day after infection during dry, warm weather, which was unfavorable to the fungus; the progress would have been much more rapid in moist weather. The black areas (*A*, *A*) represent the amount of dead tissue on the fourth day. The subsequent enlargements for the sixth, eighth, tenth, and twelfth days are indicated by the various shadings.

repeated on August 9, 12, 14, 16, 17, and 19. Well-marked differences were noted, both in number of resulting infections and in rapidity of development. Two of the leaves were lost from the Irene plants by accident.

Table X shows the percentage of infection on the remaining leaves.

TABLE X.—*Percentage of leaf infection shown by different varieties of potatoes.*

Varieties tested.	Number of leaves—		Percentage of infection.	Percentage of total leaf area found diseased on successive days after infection.				
	Inoculated.	Infected.		4 days.	6 days.	8 days.	10 days.	12 days.
Ionia Seedling	20	17	85	7.14	8.11	24.67	52.12	58.98
Holborn Abundance	20	11	55	.29	5.78	21.98	22.93	40.93
Irene	18	7	39	3.00	5.75	17.25	18.00	18.00

Had the weather been more favorable the progress of the fungus would have been more rapid, of course; but the slower progress was more favorable for our purposes, viz, the observation of the comparative progress.

These results show a well-marked resistance to fungus invasion on the part of Irene, with less by Holborn Abundance and least by Ionia Seedling. Moreover, these differences correspond very well with those recorded by Stuart (1905, p. 111) of the percentage of foliage he observed to be affected by late-blight in his field trials as a result of natural infection. In his field, when Ionia Seedling showed 20 per cent of foliage affected, Holborn Abundance showed

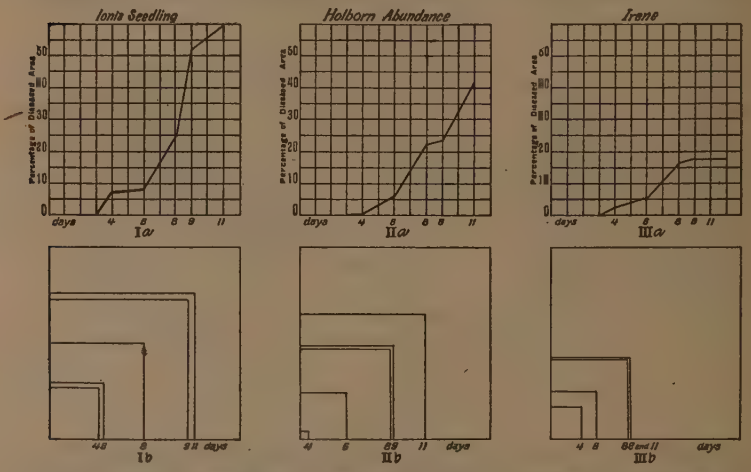

FIG. 9.—Graphic representation of the relative rates of progress of *Phytophthora infestans* following artificial infection of the leaves of three varieties of potatoes, the American variety Ionia Seedling (I*a*, I*b*) being highly susceptible, the British variety Holborn Abundance (II*a*, II*b*) moderately so, and the German variety Irene (III*a*, III*b*) highly resistant. In the upper diagrams each unit on the base line represents one day and each vertical unit 5 per cent of the area of leaf. In the lower diagrams the largest square represents the total leaf area and the smaller inclosed squares show the percentages of disease on the fourth, sixth, eighth, ninth, and eleventh successive days.

15 per cent and Irene only 5 per cent. Still more marked was the difference in the rate of progress of the disease, which was considerably the more rapid on Ionia Seedling, less on Holborn Abundance, and least on Irene. In order to compute these differences with more exactness the planimeter was used for making exact measurements of the leaf outlines and of the successive increases in blighted area. These results are also shown in Table X.

A second series of inoculations was made later in the same month. These inoculations showed essentially the same differences, but the progress of the disease was slower; and this was true of a third series

run in September, owing partly to unfavorably dry weather the latter part of the summer.

The graphic representation of the relative rates of progress of the blight shown in figure 9 is based on the figures in the first series. The results from the second series are also plotted for comparison (fig. 10). As already noted, the developments here were slower, but the relative rates were quite similar. In the first set of diagrams each unit in the base line represents one day and each unit in the vertical 5 per cent of diseased area. In the second set the largest square represents the total leaf area and the smaller inclosed squares

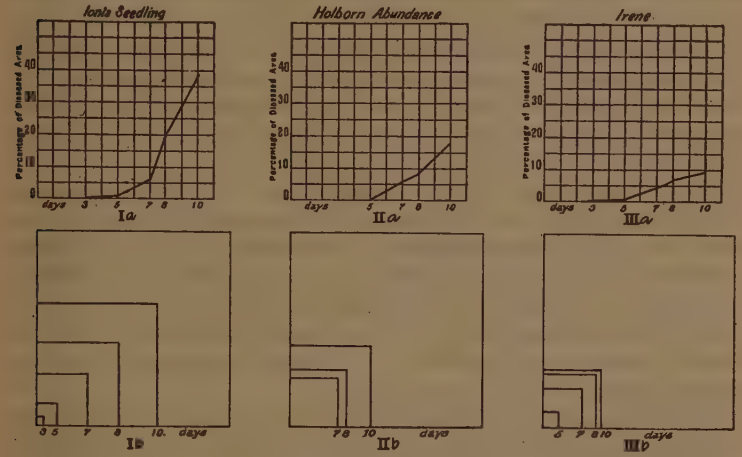

FIG. 10.—Graphic representation of the relative rates of progress of *Phytophthora infestans* following artificial infection upon potato leaves of the same varieties shown in figure 9, in a second series of experiments, the American variety, Ionia Seedling (I*a*, I*b*) being highly susceptible, the British Holborn Abundance (II*a*, II*b*) moderately so, and the German variety Irene (III*a*, III*b*) highly resistant. In the upper diagrams each unit on the base line represents one day and each vertical unit 5 per cent of the area of leaf. In the lower diagrams the largest square represents the total leaf area and the smaller inclosed squares show the percentages of disease on the successive days recorded on the margins. Comparison with the diagrams in figure 9 shows similar differences, although the rates of progress were slower.

represent the percentages of diseased areas on the successive days of observation.

Conclusions.—While these trials were too limited to justify broad generalizations, they seem clearly to show two things concerning foliage resistance: (1) That when these different potato varieties were exposed to like opportunities for foliage infection a difference appeared in the actual number of infections that resulted, indicating variations in relative Phytophthora resistance; (2) that following such infection a still more marked difference was shown in the corresponding rate of leaf-tissue destruction in the resistant as compared with the susceptible varieties. This second observation clearly indi-

cates that the disease-resisting characteristic is largely, if not wholly, inherent within the interior tissue or mesophyll of the leaf rather than in the epidermis.

STUDIES OF PHYTOPHTHORA RESISTANCE AS SHOWN BY POTATO TUBERS.

All previous studies have been based on field observations of the difference in the amount of rot following natural infection while the tubers were lying in the soil. From the inception of our work in cultivating the fungus upon the sterile blocks cut from the tubers variations were observed in the amount of growth obtained. As the work progressed systematic trials were undertaken to see how wide and constant this variation is between varieties and to perfect laboratory methods for determining such variation. At first difficulties were encountered in so preparing the potato blocks as to insure a close enough uniformity in conditions to permit of reliable conclusions. As the work progressed laboratory methods were so perfected that these difficulties were fairly well overcome.

Trials were made to obtain information on the following points: Is it practicable by the laboratory method to determine the relative disease resistance of the tubers of different varieties? Is such disease-resistant quality localized in the tuber—e. g., is it in the superficial as compared with deeper portions of the flesh? What is the basis of such disease resistance? Is it intimately associated with or inherent in the living protoplasm or is it due to some chemical content separable from the living tissue?

The tubers used for determining this point were selected, with one exception, from those grown on the trial grounds of the Horticultural Department of the Vermont experiment station at Burlington in 1909, under the direction of William Stuart, horticulturist. The single exception was the Irish Cobbler, which was grown in another trial field at St. Albans, Vt. The field where the others were grown is a uniform sandy loam which had received annually for the preceding four years 800 pounds of concentrated fertilizer per acre (200 pounds cottonseed meal, 400 pounds acid phosphate, 133 pounds muriate of potash, 67 pounds nitrate of soda). The seed tubers were from the strains grown on the trial grounds at this station under uniform conditions for four seasons previous, and the uniformity in these as well as in soil conditions leads us to believe that the variations which might result from lack of uniformity in seed or soil were largely eliminated.

RELATIVE RESISTANCE SHOWN BY DIFFERENT VARIETIES.

For these trials uniformity in laboratory method and material was the first consideration. The following details are therefore given. They will be made clearer by reference to Plates IX and X.

Uniform test tubes (13 by 1.5 cm.) were used, and 1 cubic centimeter of distilled water was placed in each together with a small wad of absorbent cotton, barely sufficient in size to hold this water. The tubes were then tightly plugged with cotton and autoclaved for 15 minutes at 5 pounds pressure. The tubes were then ready to receive for trial the sterile potato blocks which were prepared as follows: Sound, smooth tubers of medium or large size were washed and immersed for five minutes in 0.1 per cent mercuric-chlorid solution. The problem now was to cut sterile blocks of uniform size from the interior of the tuber and insert one such freshly cut, uncontaminated block into each tube. The desired result was accomplished satisfactorily after some practice by working under a hood, holding the tuber with a sterile fork and cutting it with heat-sterilized, but cool, knives. Precautions must be taken not only against germ contaminations but also against carrying any mercuric chlorid from the surface layer to the interior flesh. For these reasons the peel was first removed by a series of cuts parallel with the surface and then a thin second layer of the flesh removed before cutting out the trial blocks. In this way the outer flesh to a depth of 0.5 to 1 centimeter was discarded. If any discoloration or other evidence of unsoundness was discovered in the interior flesh the tuber was, of course, rejected. The interior flesh was then cut lengthwise of the tuber into blocks as uniform in size as practicable (about 1 by 1 by 4 cm.), each block as cut being promptly transferred to its tube. It is desirable to have the block as large as will slip easily into the tube, so as to insure uniformity in moisture conditions. A good-sized tuber will give 15 to 18 such blocks. The tubes so prepared were kept at room temperature 24 hours before inoculating, and those which showed signs of contamination were discarded. After perfecting the methods described but little trouble was experienced from contamination. To further guard against irregularities resulting from contamination as well as from minor variations in size of block, moisture relations, success in inoculation, etc., 15 blocks of each variety were used in each trial.

The inoculations in each series were made with care from pure cultures of Phytophthora grown on Lima-bean agar for 10 to 15 days to insure uniformity. It was found that uniformity in inoculation was best attained by using a stiff platinum-iridium needle with an L-shaped bend at the tip and transferring a small bit of the agar along with the fungus. It is, of course, important to have the piece transferred as uniform in size as practicable; ours were about 3 millimeters in diameter. Uniform growth was further insured by placing this piece of agar on the side of the potato block about 1 centimeter from the top and scratching it into the surface sufficiently to insure close adhesion (Pls. IX and X).

It was soon learned that the standard American variety Green Mountain is one of the most favorable for the development of the fungus (Pl. IX), while the German variety Professor Wohltmann is one of the more resistant. These two varieties were therefore taken as standards with which all other varieties were thereafter compared. It was found that about 200 tubes were as many as could be prepared and examined in one series. Fourteen varieties, 15 tubes each, were therefore started in each series, including Green Mountain, Professor Wohltmann, and 12 others. After inoculation the cultures were held in a moist incubator at 15° to 16° C. A fair growth was thus obtained by the sixth day and the maximum, on the more susceptible varieties like Green Mountain, was reached in 10 days. On the seventh day the cultures were carefully examined and the 10 tubes of each variety showing the best development were selected for further comparison with the two control varieties and with each other.

Another comparative examination was made on the tenth day, and in doubtful cases a third examination was made on the thirteenth day.

The photographs reproduced in Plates IX and X will give some notion of the differences in growth shown on certain of these varieties; the differences shown are much less marked than in the original cultures, owing to the impossibility of representing the details of the fungous mycelium, especially when photographed through the curved walls of the culture tube.

In order to establish standards for comparison, judgment was recorded on a percentage basis, the most vigorous growth, which entirely covered the surface of the potato block, being rated at 100 per cent and lesser growths in proportion. On this basis the Green Mountain was rated pretty uniformly at 95 per cent, whereas the weak growth on the control variety, Professor Wohltmann, was rated at about 10 per cent. Two persons recorded their judgment independently upon the relative developments, and three or more judges passed upon many of them. The general reliability of the method is attested by the fact that difference in these judgments was rarely noted as to the sequence in which the various varieties under inspection were to be placed; usually the percentage ratings given by the different judges were within 5 to 15 per cent of each other, and frequently they were identical.

After carrying through the first series of tests a second series was run, involving all varieties, and where the results obtained in the second series differed much from those of the first, still further tests were made. Working in this way it required the preparation and examination of some 3,000 cultures to reach conclusions as to the rating of the 76 varieties reported upon in Table XI. The final

percentage rating given in the table is the average of all ratings made upon the mature (10-day) cultures of that variety in the two or more series.

TABLE XI.—*Relative percentage of growth of Phytophthora in pure tube cultures on potato tubers of different varieties, representing five different countries.*

No.	Name of variety.	Source.[1]	Growth.	No.	Name of variety.	Source.[1]	Growth.
			Per cent.				*Per cent.*
1	Royal Kidney	Britain	1	39	Topaz	Germany	50
2	Gastold	Germany	2	40	Enormous	America	50
3	Geheimrat Thiel	do	4	41	Max Eyth	Germany	50
4	Evergood	Britain	6	42	Pearl of Carmen Valley	America	55
5	Up-to-Date	do	7	43	Million Dollar	do	55
6	Quarantaine de la Halle	France	7	44	Silesia	Germany	60
7	Eldorado	Britain	8	45	Apollo	do	60
8	Goodfellow	do	8	46	Money Maker	Britain	70
9	Magnum Bonum	do	9	47	Ninety Fold	do	70
10	Sir John Llewellyn	do	10	48	Burbank	America	70
11	Langworthy	do	10	49	Dabersche	Germany	70
12	Professor Wohltmann	Germany	11	50	Manistee	America	70
13	Irene	do	11	51	Twentieth Century	do	75
14	Radium	Britain	12	52	Gem of Aroostook	do	78
15	Malador	Holland	12	53	Rural Blush	do	78
16	Alexander's No. 1 Red	America	13	54	Dakota Red	do	80
17	Eureka	Holland	14	55	American Wonder	do	80
18	Landskroon	do	16	56	Northern Star	do	80
19	Sophie	Germany	20	57	Early Rose	do	83
20	Professor Maerker	do	20	58	June	do	85
21	Duke of York	Britain	23	59	Irish Cobbler	do	85
22	British Queen	do	25	60	Vermont Gold Coin	do	86
23	Gelbfleischige Speise	Germany	25	61	Delaware	do	87
24	Daisy	Holland	25	62	President Kruger	Germany	87
25	Jersey Peachblow	America	25	63	Beauty of Hebron	America	87
26	Richter's Imperator	Germany	25	64	Star of the East	do	87
27	Manly	America	28	65	Sir Walter Raleigh	do	90
28	Fuerst Bismarck	Germany	28	66	Rural New Yorker No. 2	do	90
29	Charles Fidler	Britain	30	67	Quick Lunch	do	90
30	Boncza	Germany	30	68	Late Blightless	do	90
31	Cambridge Russet	America	35	69	Belle de Fontenay	France	90
32	Leo	Germany	40	70	Smith's Blight proof	America	91
33	Windsor Castle	Britain	40	71	Green Mountain jr	do	91
34	Eigenheimer	Germany	45	72	Harris Snowball	do	93
35	Holborn Abundance	Britain	45	73	Norcross	do	94
36	Keeper	America	45	74	Early Excelsior	do	94
37	Factor	Britain	47	75	Quick Crop	do	94
38	Mohort	Germany	50	76	Green Mountain	do	95

[1] The original source of the variety is stated, viz, whether its origin is Great Britain (Britain), Germany, France, Holland, or America. Tubers were selected from strains which had been grown under uniform conditions upon a sandy loam on the trial grounds of Prof. William Stuart, horticulturist of the Vermont Agricultural Experiment Station. Moreover, most of them had been similarly grown for four seasons previously. All of the foreign strains—British, German, French, and Dutch—were obtained from reliable parties in their respective European countries in 1904 and there selected because of their reputed disease-resistant qualities. Most of the American varieties were assembled that same year as including the most promising American varieties with respect to disease resistance. For further details as to the assembling of these, their exact source, and their reputed disease-resistant qualities, see Jones, 1905, pp. 28–39; Stuart, 1906, pp. 134–136.

Inasmuch as the basis of the percentage rating was established somewhat arbitrarily, the chief significance of the table lies in the sequence of the varieties. Further trials, especially if made with tubers from different sources, might lead to minor changes in their sequence. It would perhaps give less opportunity for misinterpretation and serve most practical results quite as well to merely divide the potatoes into five groups marked by the 20, 40, 60, and 80 per cent ratings given above.

Group 1. *Highly resistant varieties:* Royal Kidney, Gastold, Geheimrat Thiel, Evergood, Up-to-Date, Quarantaine de la Halle, Eldorado, Goodfellow, Magnum Bonum, Sir John Llewellyn, Lang-

worthy, Professor Wohltmann, Irene, Radium, Malador, Alexander's No. 1 Red, Eureka, Landskroon, Sophie, Professor Maerker (9 British, 7 German, 2 Dutch, 1 French, 1 American).

Group 2. *Moderately resistant:* Duke of York, British Queen, Gelbfleischige Speise, Daisy, Jersey Peachblow, Richter's Imperator, Manly, Fuerst Bismarck, Charles Fidler, Boncza, Cambridge Russet, Leo, Windsor Castle (3 British, 6 German, 1 Dutch, 3 American).

Group 3. *Intermediate:* Eigenheimer, Holborn Abundance, Keeper, Factor, Mohort, Topaz, Enormous, Max Eyth, Pearl of Carmen Valley, Million Dollar, Silesia, Apollo (2 British, 6 German, 4 American).

Group 4. *Moderately susceptible:* Money Maker, Ninety Fold, Burbank, Dabersche, Manistee, Twentieth Century, Gem of Aroostook, Rural Blush, Dakota Red, American Wonder, Northern Star (2 British, 1 German, 8 American).

Group 5. *Very susceptible:* Early Rose, June, Irish Cobbler, Vermont Gold Coin, Delaware, President Kruger, Beauty of Hebron, Star of the East, Sir Walter Raleigh, Rural New Yorker No. 2, Quick Lunch, Late Blightless, Belle de Fontenay, Smith's Blightproof, Green Mountain Jr., Harris Snowball, Norcross, Early Excelsior, Quick Crop, Green Mountain (1 German, 1 French, 18 American).

The first group is the one in which the minor differences are of chief practical interest. It is, therefore, of importance to note that it was in this one that the ratings were most easily and confidently decided and in which we believe they have the most permanent value.

The remarkably high degree of disease resistance which occurs in certain of the European varieties as contrasted with the American varieties is clearly shown in Table XI. It is to be remembered that these European varieties were selected by the senior writer in 1904 for reputed disease resistance, and their relative merits in this respect were demonstrated soon thereafter by Stuart (1906). Stuart's results were derived from field trials using these same varieties and the same strains in practically all cases which we have since been using for these laboratory trials. It is of especial interest, therefore, to compare his results with those recorded above. These are entered in Table XII for all the varieties which were included in both series of trials. Stuart's records show the amount of rot developed in the field under natural conditions of infection associated with an epidemic of late-blight of the foliage. His trials were conducted on two fields, (A) a sandy-loam soil and (B) a clay loam. There was much rain in the autumn, when Phytophthora was prevalent, and as a result there was much more rot on the clay. The first figure column in the table shows the percentage rating given in our trials, the second column the percentage of rot which occurred in Stuart's trial

on the sandy field, and the third column the percentage of rot in Stuart's clay field.

TABLE XII.—*Development of Phytophthora in laboratory trials compared with the percentage of rot observed in the field on different varieties of potatoes.*

Variety.	Laboratory trial.	Field trial.		Variety.	Laboratory trial.	Field trial.	
		Sand.	Clay.			Sand.	Clay.
Royal Kidney	1	2	12	Eigenheimer	45	1	21
Gastold	2	1	9	Holborn Abundance	45	10	38
Geheimrat Thiel	4	4	18	Keeper	45	2	12
Evergood	6	1	3	Factor	47	17	60
Up-to-Date	7	6	38	Mohort	50	0	4
Quarantaine de la Halle	7	8	83	Topaz	50	1	.....
Eldorado	8	1	0	Enormous	50	23	68
Goodfellow	8	1	3	Max Eyth	50	2	20
Magnum Bonum	9	1	16	Million Dollar	55	16	42
Sir John Llewellyn	10	0	72	Silesia	60	1	10
Langworthy	10	1	19	Apollo	60	0	5
Professor Wohltmann	11	1	1	Money Maker	70	5	.....
Irene	11	1	0	Dabersche	70	1	49
Radium	12	5	.....	Gem of Aroostook	78	16	77
Malador	12	1	9	Rural Blush	78	5	56
Alexander's No. 1 Red	13	1	1	Dakota Red	80	4	40
Eureka	14	1	5	American Wonder	80	2	42
Landskroon	16	2	11	Northern Star	80	2	27
Sophie	20	2	9	Early Rose	83	19	89
Professor Maerker	20	4	8	June	85	.....	30
Duke of York	23	4	78	Vermont Gold Coin	86	22	74
British Queen	25	5	33	Delaware	87	18	73
Gelbfleischige Speise	25	1	27	Star of the East	87	27	55
Daisy	25	0	9	Sir Walter Raleigh	90	21	62
Richter's Imperator	25	4	29	Rural New Yorker No. 2	90	6	45
Fuerst Bismarck	28	1	4	Quick Lunch	90	9	.....
Charles Fidler	30	0	.....	Late Blightless	90	7	34
Boncza	30	2	11	Belle de Fontenay	90	3	49
Cambridge Russet	35	2	60	Harris Snowball	93	9	73
Leo	40	0	18	Norcross	94	29	68
Windsor Castle	40	3	30	Green Mountain	95	21	80

Of course, the basis for these figures is so entirely different that any detailed numerical comparison is idle. The relative rating in each trial is the thing of prime significance. As already stated, the chief interest centers upon the detection of varieties of especial disease-resistant quality. Stuart's trials on the sandy field showed extreme percentages of rot, ranging from zero to about 30. In the clay field the extreme reached 100 per cent. If we are to attempt, somewhat arbitrarily it is true, to make five groups upon the basis of Stuart's trials, as was done upon the basis of our trials, it would seem that all varieties showing less than 10 per cent of rot in the sandy field might properly be rated, so far as that evidence goes, as highly disease resistant, and those showing less than 20 per cent rot in the clay fields should be thus rated. Scanning the figures given for the 20 varieties rated on the basis of our tests as in the highly disease-resistant class, it will be seen that all but two, or perhaps three, are put in the same group by Stuart's figures. The exceptions are Up-to-Date, which according to Stuart's figures would go into the second group, and Quarantaine de la Halle, which showed a still higher per-

centage of rot and would be by these figures rated as a susceptible variety. The Sir John Llewellyn showed considerable rot on the clay soil but none whatever on the sandy soil. Both the Up-to-Date and the Sir John Llewellyn varieties are reported as highly disease resistant in Great Britain.

So far as can be judged from the data at hand, therefore, this laboratory method seems reliable as well as expeditious for the determination of the Phytophthora-resistant qualities of the tubers. It possesses certain advantages which are at once obvious over the method of field trial, depending as that does on the chances of natural infection. The natural infection of the tubers in the field is conditioned in the first place upon the development of the fungus on the foliage. This is by no means uniform even on the same variety and varies widely on different varieties. Moreover, according to Stuart's observations (1906, p. 116) foliage resistance is not always correlated with tuber resistance. Thus the American variety Rust-proof showed a high degree of foliage resistance, but the tubers are especially subject to rot. Apollo, a German variety, showed only 5 per cent of leaf infection in Stuart's trials, as compared with 95 per cent on the English variety Royal Kidney. Our laboratory tests show Royal Kidney tubers to be much more resistant than Apollo. Stuart's field trials on the other hand show more rot in Royal Kidney than in Apollo. It seems reasonable to believe that this variation in results may have been due merely to the difference in natural infection resulting from greater development of the fungus on the Royal Kidney foliage. Minor variations in character of soil and surface drainage may have a marked effect on the percentage of rot, as may also the depth to which the tubers are buried. Moreover, if one depends wholly on field trials he must often wait several years before climatic conditions are such as to bring on the sudden and uniform development of the fungus which is necessary to furnish satisfactory data. On the other hand, by the laboratory method the relative Phytophthora resistance of a given series of tubers can be determined in a short time quite independently of the relation to foliage, the changes of climate, or the variations in soil.

The data already given, comparing the resistance of the several varieties to Phytophthora in the laboratory tests with the results in the field, have been arranged by Mr. J. B. Norton in the form of a table showing correlation for the sandy field and for the clay field.

TABLE XIII.—*Correlation between percentage of infection of tubers of potatoes with Phytophthora in laboratory tests and percentage of rot in tubers of same varieties grown on sandy soil or on clay soil.*

Percentage of rot of tubers grown on—	Number of varieties showing given percentages of infection in laboratory tests.										Frequency.	Departure from mean.
	0 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60	61 to 70	71 to 80	81 to 90	91 to 100		
Sandy soil:												
0 to 3	8	7	5	3	5	2	1	2	1	.....	34	− 4.5
4 to 6	3	2	3	.....	.....	.....	1	2	1	.....	12	− 1.5
7 to 9	1	.....	.....	.....	.....	.....	.....	.....	2	1	4	+ 1.5
10 to 12	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	1	+ 4.5
13 to 15	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	+ 7.5
16 to 18	.....	.....	.....	.....	1	.....	.....	1	1	1	4	+10.5
19 to 21	.....	.....	.....	.....	.....	1	.....	.....	2	.....	3	+13.5
22 to 24	.....	.....	.....	.....	1	.....	.....	.....	1	.....	2	+16.5
25 to 27	.....	.....	.....	.....	.....	.....	.....	.....	1	.....	1	+19.5
28 to 30	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	1	+22.5
Frequency	12	9	8	3	8	3	2	5	9	3	62	
Departure from mean	−33.4	−23.4	−13.4	−3.4	+6.6	+16.6	+26.6	+36.6	+46.6	+56.6	.....	
Clay soil:												
0 to 10	4	7	2	.....	1	2	.....	.....	.....	.....	16	−30
11 to 20	4	1	1	1	2	.....	.....	.....	.....	.....	9	−20
21 to 30	.....	.....	2	1	1	.....	.....	1	1	.....	6	−10
31 to 40	1	.....	1	.....	1	.....	.....	1	1	.....	5	0
41 to 50	.....	.....	.....	.....	.....	1	1	1	2	.....	5	+10
51 to 60	.....	.....	.....	1	1	.....	.....	1	1	.....	4	+20
61 to 70	.....	.....	.....	.....	1	.....	.....	.....	1	1	3	+30
71 to 80	1	.....	1	.....	.....	.....	.....	1	2	2	7	+40
81 to 90	1	.....	.....	.....	.....	.....	.....	.....	1	.....	2	+50
Frequency	11	8	7	3	7	3	1	5	9	3	57	
Departure from mean	−40	−30	−20	−10	0	+10	+20	+30	+40	+50	.....	

Coefficient of correlation between laboratory tests and tests on sandy field, 0.5936±0.055; between laboratory tests and tests on clay field, 0.584±0.059.

Mr. Norton appends a paragraph of explanation, as follows:

In attempting a statistical study of the results presented in Table XIII it becomes at once apparent that the varieties used in the test were not selected at random in regard to their resistance to the rot. The European varieties, in particular, were the most rot resistant that could be found. In neither of the field trials nor in the laboratory test do the frequency distributions suggest a normal probability curve, but this fact does not seriously affect the results. The important fact shown in the table is that in very few cases the rot in the field trials exceeds in a marked degree the susceptibility indicated by the laboratory test. This fact makes the reliability of the laboratory test much higher than the correlation indicates. The tubers used in the field trials in many cases failed to rot where the laboratory test shows susceptibility. These cases, while weakening the coefficient of correlation, do not affect the value of the method, since in most cases this failure to rot is probably due to environmental conditions preventing infection in the field trials.

NATURE AND DISTRIBUTION OF THE PHYTOPHTHORA-RESISTING PROPERTY.

Early in the conduct of these studies the question arose as to the location or distribution of the disease-resistant quality and its possible nature. Various lines of study with both foliage and tubers

were undertaken to obtain data as to these matters. So far as these studies relate to the fundamental question of the nature of the disease resistance the results are largely negative, but certain facts were learned as to its distribution.

The trials already discussed show that with both organs the fungus resistance is not merely due to difference in epidermal or superficial tissues. Thus in Irene the progress of the fungus through the mesophyll of the leaf was much slower than with Ionia Seedling, showing that there is something in the interior tissues of Irene which furnishes a Phytophthora-resistant character quite apart from any possible differences in epidermal characters. With the tubers, likewise, our trials show that there is a well-marked difference in fungus resistance resident in the living interior flesh of certain varieties and uniformly distributed through this flesh. This does not prove that there is no difference due to the character of the surface tissues or skin of the potato, as has been advocated by Sorauer (1902). But inasmuch as neither our results nor those of Stuart show any such relation to exist and in view of the evidence previously cited that tuber infection occurs chiefly through breaks in the corky covering, i. e., eyes, wounds, or lenticels, it seems probable that the disease-resistant quality of the tuber is resident entirely within the flesh and quite independent of the character of the skin.

Having decided that the fungus-resistant character is resident within the interior tissues of the leaves and tubers of certain varieties, the attempt was made to learn more as to its nature. Inoculation experiments had already shown the foliage of Irene to be highly resistant, that of Ionia Seedling proved especially susceptible, while Holborn Abundance and Dakota Red were intermediate in resistant qualities. The juice was extracted from these leaves and its degree of acidity determined by titration. That of Irene was found to be +10.6 Fuller's scale; Ionia Seedling, +12.2; Holborn Abundance, +8; and Dakota Red, +14.8. There was no indication, therefore, of any direct relation between the reaction of the juice and the disease-resistant quality of the tissue from which it was extracted. Further evidence on this point was sought by taking the juice from the leaves of Holborn Abundance, normally having the reaction +8 Fuller's scale and dividing it into three parts. One part was made neutral and a second part −8 by the addition of sodium hydroxid. These parts were then sterilized by heating in the steamer for three consecutive days and inoculated with Phytophthora. The growth in all was slow and small in amount. The difference was slight, but was less in the more alkaline (−8) tube than in the others. So far as this result had any significance, therefore, it indicates that the disease-resistant quality was not due to the acidity differences.

The question arose as to whether the resistant quality is still retained in the extracted juice. To determine this a quantity of the juice was expressed from the leaves of each of the three varieties, Irene, Dakota Red, and Ionia Seedling. These were separately passed by suction through Pasteur-Chamberland filters in order to render them sterile and were placed in sterile tubes. These tubes were then inoculated with Phytophthora and held at 18° to 20° C. for some three months. The growth in all the tubes was slow and small in total amount. There was no difference of significance between the amount of the growth in the different juices. Since Irene and Ionia Seedling represent extremes in natural disease resistance the conclusion reached was that the disease-resistant quality is either inseparably associated with the living tissues or else was retained in the mesh of the filter. Experience has shown that enzymes originally in solution may be removed by porcelain filters (Jones, 1909, p. 298). In order further to test this matter the juice was similarly extracted separately from the raw tubers of these same varieties, Irene, Dakota Red, and Ionia Seedling. Part of this juice was simply passed through the Pasteur-Chamberland porcelain filters, part was filtered and in addition heated in the steamer for 15 minutes on each of three successive days, and part of the latter was in turn filtered a second time so as to remove the coagulum resulting from heating. The purpose was to learn if the resistant principle might be destroyed or removed by either the heating or the filtration. Here again the growth was small in all tubes. Since, however, there was no difference of significance as between the different varieties it seems that any disease-resistant property possessed in these original juices was lost in the treatment. Like trials were made with juices expressed from tubers of these varieties which were simply sterilized by heating in the steamer on three consecutive days. These tests also showed no differences of note. Finally tubers of each of the three varieties Irene, Dakota Red, and Ionia Seedling were used in the preparation of potato gelatin, according to the method outlined earlier in this bulletin (p. 47). Here again the growth was essentially alike on the media from all three varieties.

Conclusions.—In view of these results it seems justifiable to conclude that well-marked and fixed differences exist among potato varieties in relative susceptibility to invasion by *Phytophthora infestans;* in other words, in disease resistance. These differences occur in foliage as well as in tuber. While foliage and tuber resistance generally go together, this relation is not invariable. The disease-resistant quality is resident in large measure, and probably wholly, in the interior tissues of both leaf and tuber. In the tuber it is uniformly distributed throughout the flesh. Variations in disease re-

sistance are not correlated with variations in acidity of the tissues. The potato tuber contains but little tannin, and Cook's results indicate that tannin content is not a factor in Phytophthora resistance in the potato. While this resistance may be and indeed probably is due to some chemical product, up to the present time efforts to retain this product, either in the cooked potatoes or in the cooked or filtered juice extracted from the disease-resisting potato varieties, have not succeeded. This product may, therefore, be assumed to be either a compound, modified or destroyed by cooking and weakened or removed by filtration through porcelain, or else it may be so intimately associated with the living protoplasm as to be inseparable from it by the processes employed.

SUMMARY.

1. The potato disease termed the late-blight and rot has been known as the most serious of all potato diseases in Europe and America since about 1845, when its outbreak was the immediate cause of the Irish famine. In the State of New York alone a loss of 20,000,000 bushels in one year was attributed to this disease, and the percentage of loss may be even greater elsewhere.

2. The disease does not attack the leaves, as a rule, until after the blossoming period, i. e., in late summer; if present and weather conditions favor, it quickly causes late-blight, which kills the foliage and thence passes to the tubers, causing the dry-rot.

3. The disease is common in the Northeastern States, being favored by rather cool, moist summers. Farther south and west it is less common, probably only occurring locally when introduced with seed from the North. It does not long persist where the late summers are warm and dry.

4. It is liable to confusion with such leaf diseases as the early-blight (Alternaria) and leaf-blotch (Cercospora) and with various types of tuber rot.

5. It is caused by the fungus *Phytophthora infestans*.

6. The fungus develops first on the foliage, from which it passes by means of spores that are washed into the soil to the tubers, in which it hibernates.

7. Jensen showed the possible efficacy of two remedial measures: (a) Burying the tubers to a sufficient depth (about 4 to 5 inches) with soil to prevent the infection; (b) disinfecting tubers designed for seed purposes by exposure to dry heat, 40° C. (104° F.) for four hours. Neither of these methods has become established in practice.

8. Studies of infection, dissemination, and disease control have shown:

(a) Tuber infection in the field may be prevented by spraying the soil even when the fungus is allowed to develop unchecked on the

foliage. This is explainable only on the assumption that the primary tuber infection comes from spores washed through the soil.

(b) Tubers may also be infected from contact with blighting foliage at digging time.

(c) Secondary infection of tubers may occur either in the soil before digging or in the storage bin from spores developed on the surface of earlier infected tubers.

(d) When the tops are attacked by late-blight the harvesting of the tubers should be delayed until a week or more after the death of the tops. Longer delay does no harm unless the season be wet and the soil exceptionally heavy.

(e) Dry, cool storage is of primary importance, the use of lime or formalin disinfection for the tubers being valueless.

(f) Wind and water are probably the important agencies in local spore distribution, but leaf-eating insects also function and may carry the spores longer distances.

(g) Spraying the foliage with Bordeaux mixture has proved an almost complete remedy against both the Phytophthora blight and the rot, and also operates beneficially to the potato plant in other ways. Spraying experiments with this mixture have been made annually at the Vermont experiment station for 20 years, 1891 to 1910, on late or main-crop potatoes, three applications generally being made. The results were an increased yield in every case, ranging from 18 to 215 per cent. The average of the yields of the 20 years on the sprayed areas was 268 bushels per acre as compared with 163 bushels on the unsprayed, a gain of 105 bushels per acre, or 64 per cent.

9. *Phytophthora infestans* has been carried in pure culture since 1904, and some strains have been thus grown continuously for over five years without evidence of change in pathogenicity or other characters. It grows best on blocks cut from the interior of raw potato, very well on Lima-bean agar and potato gelatin, and also, as recently shown by Clinton, on oat agar. It has been grown, but with less vigor, on various other vegetables and synthetic media.

10. The fungus in culture will survive a fairly wide range in the reaction of the medium. Fructification was checked by an alkalinity of −3 Fuller's scale and by an acidity of +15. Vegetative growth occurred between −8 and +25. These facts are of possible interest in connection with the relation of the fungus both to the host tissues and to fungicides.

11. Exposing test-tube cultures for 10 minutes at temperatures up to 40° C. did not prevent the later development of the fungus; beyond this temperature inhibition resulted. Where cultures were held at constant temperatures the best growths resulted between 16° and 19° C. Below 16° C. the growth was slower, and below 5° C. it was

wholly inhibited. At and above 23° C. the growth was inhibited, with no sporulation above 25° and no vegetative growth at or above 30°.

12. Bodies having the characters of thick-walled spiny resting spores were produced in pure cultures in potato gelatin and in Lima-bean agar. In the early stages of their development these bodies had the general appearance and the cytological characters of oogonia, but no antheridia were found, and they apparently developed asexually. Nine strains of the fungus from widely different sources in America and Europe have been studied with reference to the production of these bodies. They were found in all. Growth in mixed culture did not increase or modify them. Persistent search failed to reveal the mature stages of these bodies in cultures upon blocks cut from potato tubers, although what were considered to be immature stages of the same bodies were found sparingly. No such bodies have been found in decaying potato tubers following Phytophthora attacks. Examination of potato leaves killed by Phytophthora showed that somewhat similar bodies occur. Some of them clearly originated from secondary saprophytes and probably all so originated. No opinion is therefore justified as to the occurrence of such resting spores in nature, although their appearance in the culture tubes proves that Phytophthora is capable of developing such bodies. In no case have these spores been seen to germinate.

13. Inoculations of potato leaves of different varieties with Phytophthora cultures showed that certain varieties are much more susceptible than others. This difference was shown in the rate of progress through the leaves following infection as well as in the number of leaves infected. This fact indicates that the disease-resistant quality is largely, if not wholly, resident within the mesophyll and that it is independent of epidermal characters.

14. Inoculations of sterile blocks cut from the flesh of potato tubers with Phytophthora cultures showed a wide range in vigor of growth, i. e., in Phytophthora-resisting properties. Blocks cut from different parts of the same tuber, and also from different tubers of the same variety, gave essentially like results. There was much difference, however, as between varieties. This difference was sufficiently marked to permit of their being graded on a percentage basis with a fair degree of assurance and to enable one to distinguish with full confidence between the especially disease-resistant varieties and those that were more susceptible.

15. A comparison of the conclusions reached by this laboratory method, using pure cultures for determining the relative disease-resistant quality of potato tubers, with the amount of tuber rot recorded by Stuart from field trials of the same varieties gives ground for confidence in its reliability as a method of judging of the rot-

resistant qualities of the variety under trial. It is a method having at least much value as supplementing field trials for the determination of disease resistance, if not of replacing them entirely.

16. These studies confirmed the conclusions reached by Stuart from his field trials that certain standard European varieties are much more highly resistant to Phytophthora attacks than are the standard American varieties.

17. Efforts to determine more definitely the location or nature of the disease-resisting property gave little that was definite beyond the fact that it was resident within the flesh of the living potato tuber and uniformly distributed through it. The difference was not correlated with differences in the relative acidity of the sap or in any other chemical factor determined and it apparently was lost with the extraction of the juice and with cooking.

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DESCRIPTION OF PLATES.

PLATE I. Potato leaf badly attacked by the late-blight fungus *Phytophthora infestans*. (From a painting by Mrs. W. J. Morse.)

PLATE II. Potato tuber with the sunken dark areas characteristic of Phytophthora infection, the condition commonly known as dry-rot. (From a painting by Mrs. W. J. Morse.)

PLATE III. Phytophthora-infected tubers with the fungus fruiting on their surfaces. Microscopic examination showed the white spots to consist of *Phytophthora infestans*, richly sporulating. (*a*) Tuber just as dug from the soil with several such fungous tufts. (*b*) Tubers from storage cellar still more abundantly studded with Phytophthora outgrowths.

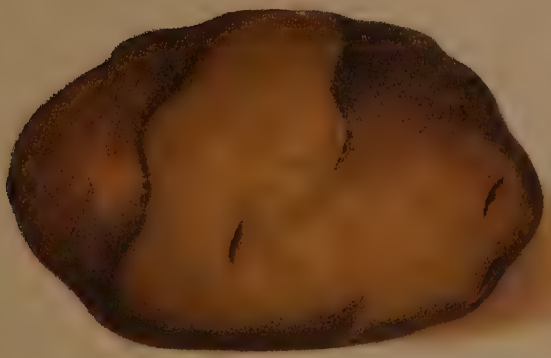
PLATES IV–VIII. Drawings of *Phytophthora infestans*. All figures in these plates, unless otherwise specified, were drawn by the aid of a camera lucida from material grown in pure culture. Figs. 1–33*b* are magnified about 500 times. Figs. 29–53 are magnified 1,000 times, except No. 48, which is 2,000 times. Figs. 1–16.—These 16 illustrations represent different types and stages in the development of the resting bodies found in our earlier potato-gelatin cultures. Some of these bodies are brown walled, while others, such as Nos. 8, 11, and 12, have a gelatinous and practically transparent outer covering, usually very much swollen, inclosing a dark-brown inner wall (figs. 5, 6, and 7). Fig. 16 shows the condition and also the granular halo in the gelatin which enveloped the outer coating. Some of the others, such as Nos. 3, 8, 10, 11, 12, and 13, show this granular layer more diffused or developed in a less marked degree. Figs. 17–23.—Clear-walled, apparently immature resting spores such as were abundant in our later culture. It will be noticed that these bodies are usually formed apically, or as side branches, but may be formed intercalary in the hyphæ. Fig. 19 shows the general resemblance of at least some of these bodies to the conidia. Figs. 25 and 26.—Clear-walled spores, but with inner portion of wall tinged with brown. Spines are apparently beginning to form in the interior. Figs. 27 and 28.—Brown-walled spores obtained from teasing Phytophthora-infected potato leaves, probably saprophytic intruders. Figs. 29–38.—Thick, brown-walled, spiny resting spores, such as have been found in our later old potato-gelatin cultures. Fig. 33*a* is a surface view of such a spore, while fig. 33*b* is a median optical section of same. Figs. 39, 40, and 41.—Clusters from potato-gelatin culture. No. 40 is clear walled; No. 39 has dark-brown, almost smooth walls; No. 41, spiny dark-brown walls. These three clusters were obtained from one slide and evidently represent different stages or types of development. Figs. 42–46.—Stained sections of immature resting spores, showing the nuclei and the peculiar structures suggestive of aborted oospheres. Fig. 47.—Details of section of one of these interior bodies. Fig. 48.—Some of the mycelium from the pure cultures showing the nuclei. Fig. 49.—Conidia stained to show nuclei. Fig. 50.—Conidium cut up into zoospores; stained to show nuclei. Fig. 51.—Zoospores stained on slide to show nucleus in each. The cilia are not stained. Fig. 52.—Zoospore which has not escaped from conidium, putting out a germ tube; nucleus stained. Fig. 53.—Haustorium from mycelium in potato tuber penetrating host cell. Drawing from a preparation courteously furnished by Dr. Delacroix.

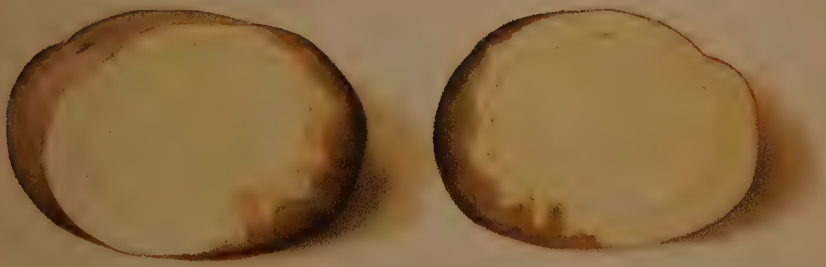
PLATE IX.—Tube cultures, 10 days old, of *Phytophthora infestans* on raw-potato blocks, showing different degrees of susceptibility or disease resistance. Tubes 1*a* to 1*d*, Irene; tubes 2*a* to 2*d*, Green Mountain. By comparison with Table XI (p. 77) it will be seen that the Irene represents the highly disease-resistant varieties (group 1, p. 77). It is not, however, the most highly resistant of these, its average growth being rated at 11 per cent (p. 77). Green Mountain is representative of the highly susceptible varieties (group 5, p. 78), showing an average growth rated at 95 per cent. Hundreds of such cultures have been made of these two varieties, using scores of tubers and from the crops of two seasons, with similar results.

PLATE X.—Cultures similar to those of Plate IX, showing four varieties: Tubes 3*a* and 3*b*, Smith's Blightproof, a highly susceptible American variety representing group 5. The next two varieties—Jersey Peachblow and Manly—also American, represent the moderately resistant varieties (group 2). Up-to-Date is a highly resistant English variety representing group 1. (For further details see text under "Disease resistance of potatoes," p. 69.)

BREUKER & KESSLER CO. PHILA

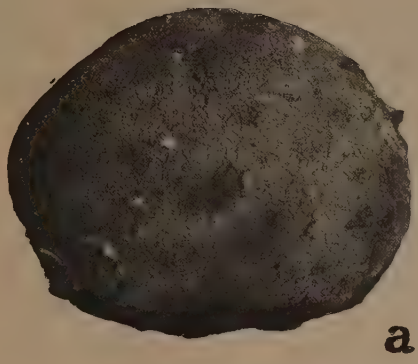
POTATO LEAVES SHOWING LATE-BLIGHT CAUSED BY PHYTOPHTHORA.

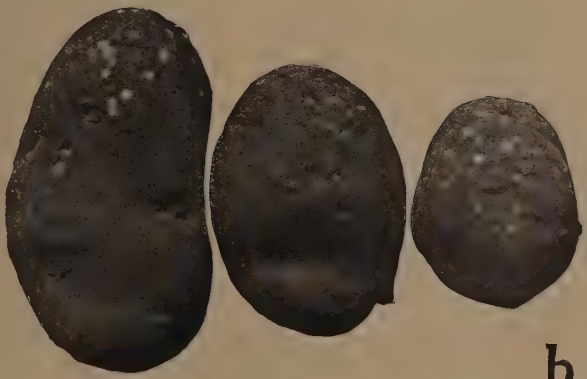




BREUKER & KESSLER CO. PHILA

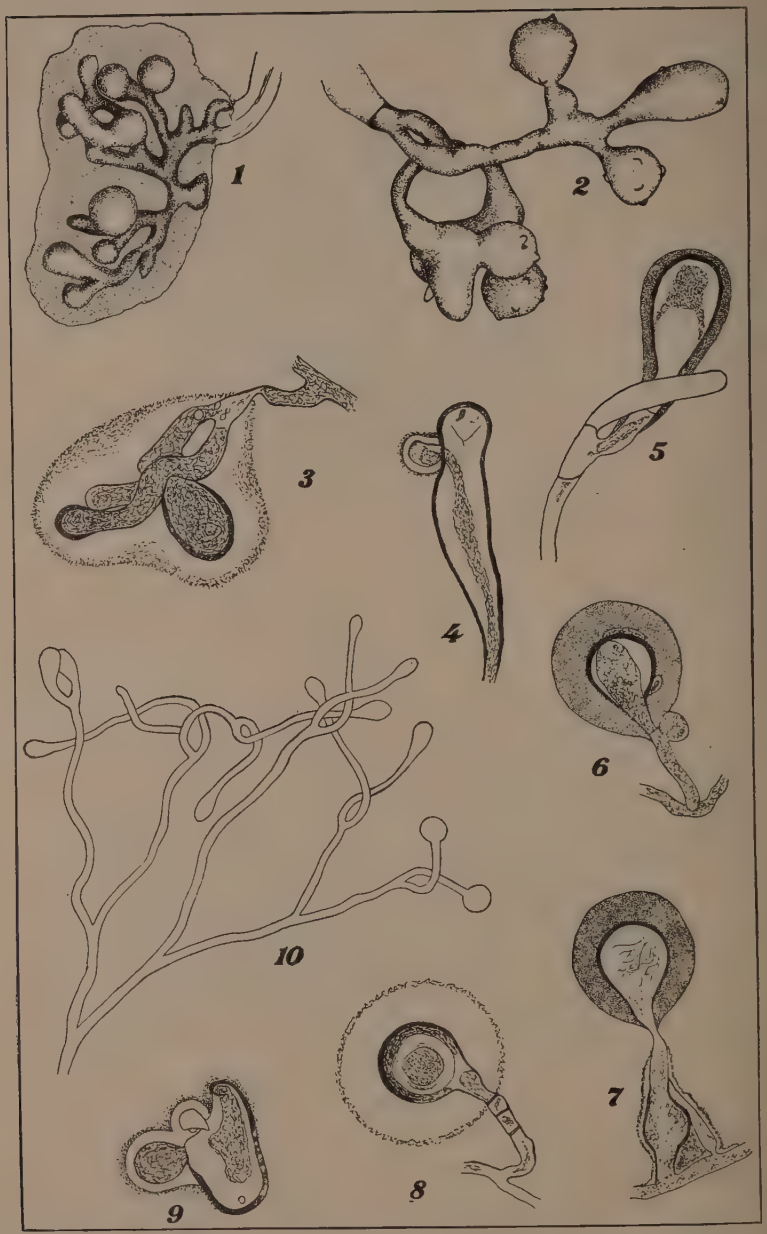
POTATO TUBER SHOWING DRY-ROT CAUSED BY PHYTOPHTHORA.





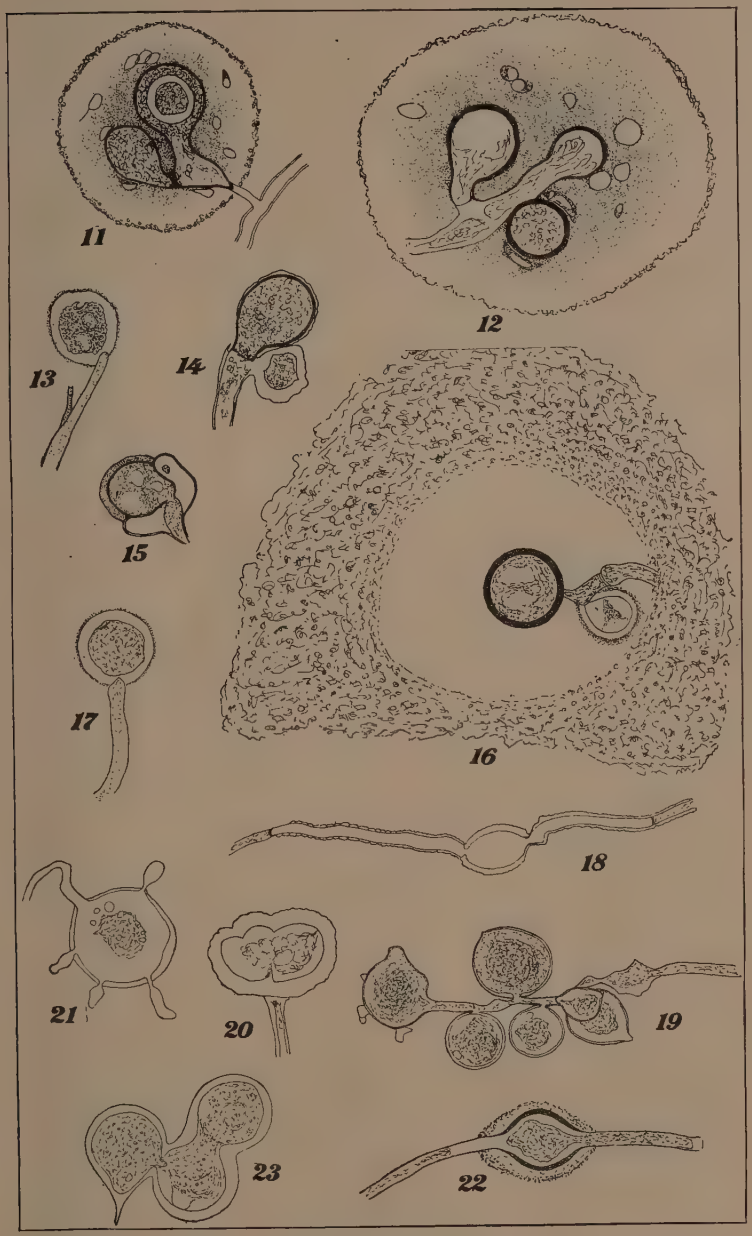
POTATO TUBERS FROM SOIL AND STORAGE, SHOWING WHITE TUFTS OF RICHLY SPORULATING PHYTOPHTHORA INFESTANS DEVELOPING ON THE SURFACE.

[The upper one (*a*) was freshly dug from the field; the lower three (*b*) were from the storage bin in the autumn. About two-thirds natural size.]



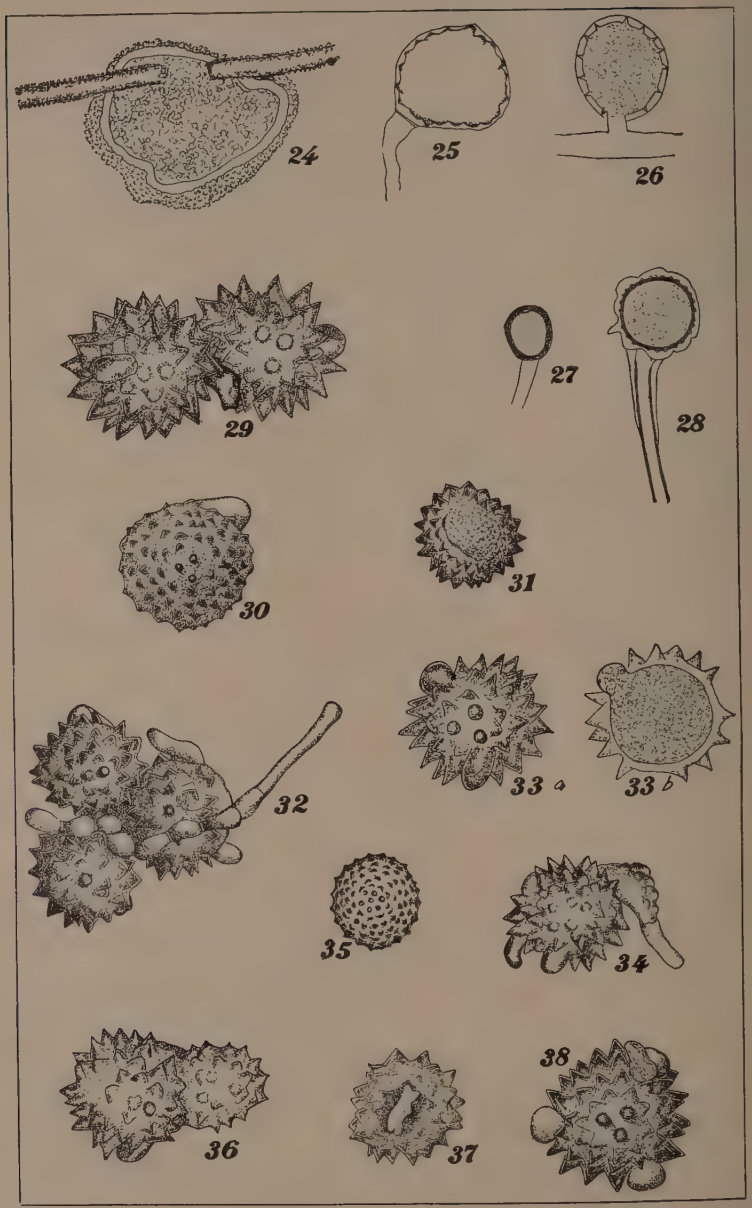
DIFFERENT TYPES SHOWN IN THE EARLY STAGES OF DEVELOPMENT OF THE RESTING BODIES AS FOUND IN PURE CULTURES OF PHYTOPHTHORA INFESTANS IN POTATO GELATIN.

[Magnified about 500 diameters, except figure 10, which is magnified about 200 diameters. For details see "Description of plates" and text.]



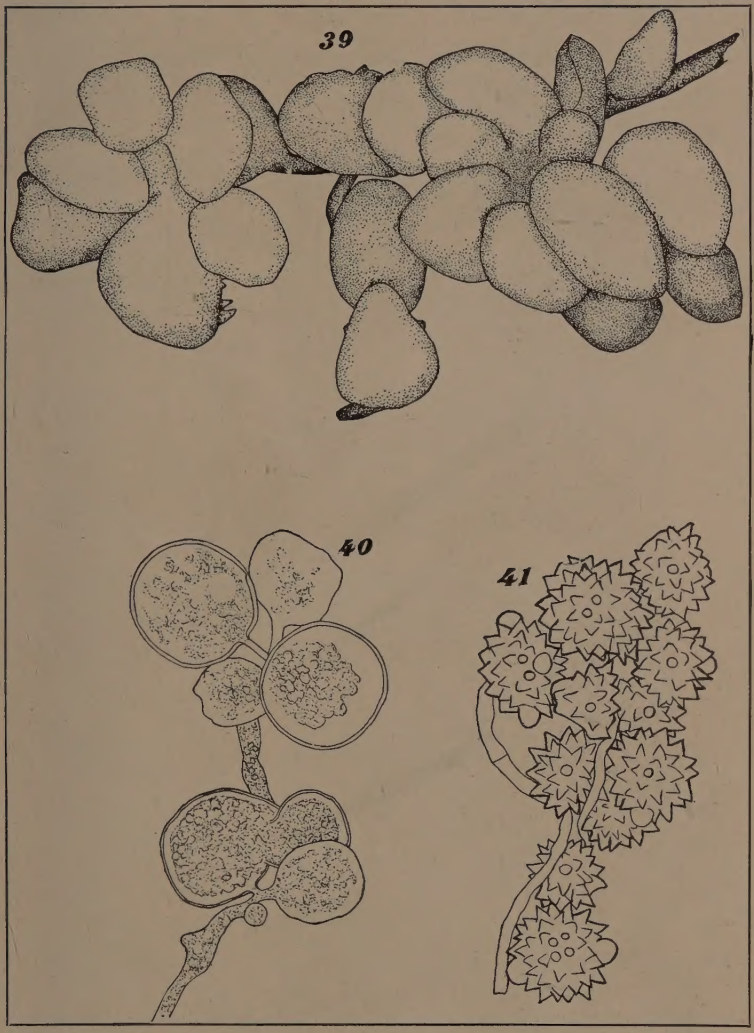
IMMATURE STAGES OF RESTING BODIES FOUND IN POTATO-GELATIN CULTURES OF PHYTOPHTHORA INFESTANS.

[For details see "Description of plates" and text.]



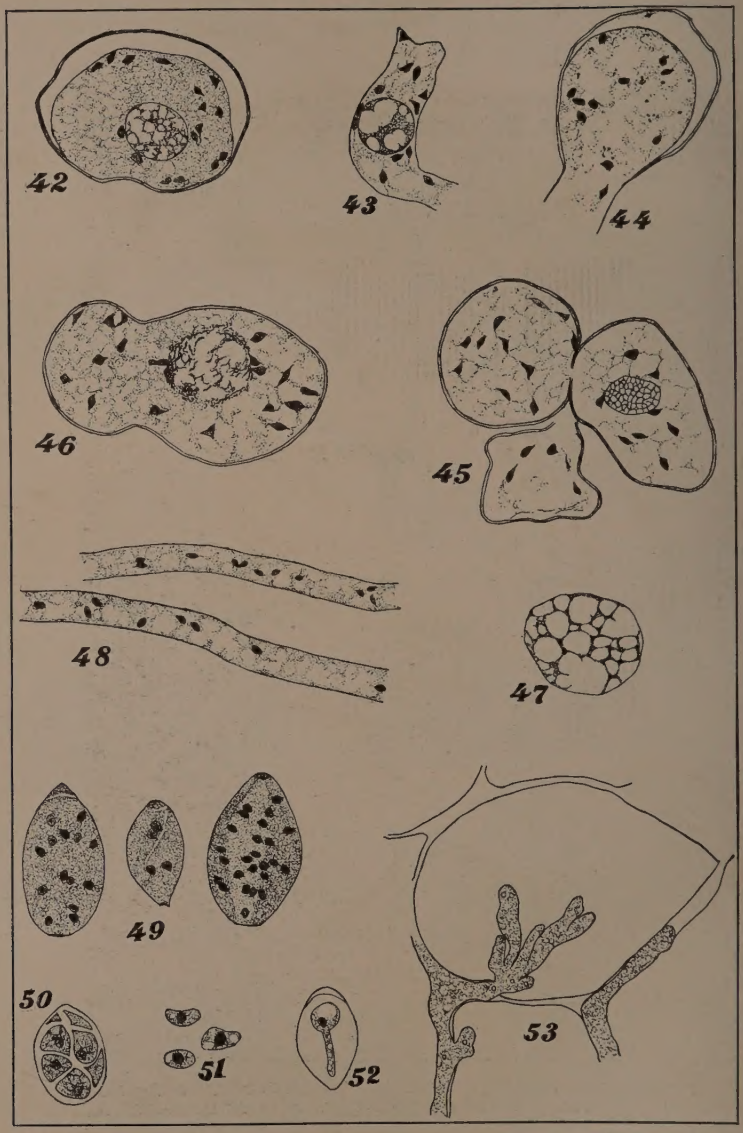
RESTING SPORES FROM POTATO-GELATIN CULTURES OF PHYTOPHTHORA INFESTANS AND SIMILAR BODIES FROM DECAYING POTATO LEAVES.

[Figs. 24–26, immature stages; figs. 29–38, mature stages of resting spores; figs. 27–28, similar bodies found in decaying potato leaves following their invasion by *Phytophthora infestans*,, but considered to be secondary saprophytic organisms.



LARGE CLUSTERS OF SPORES FROM POTATO-GELATIN CULTURES OF PHYTOPHTHORA INFESTANS, SHOWING DIFFERENT STAGES OF DEVELOPMENT.

[Figs. 39 and 40, immature; fig. 41, mature; all from the same slide. For details see "Description of plates" and text.]

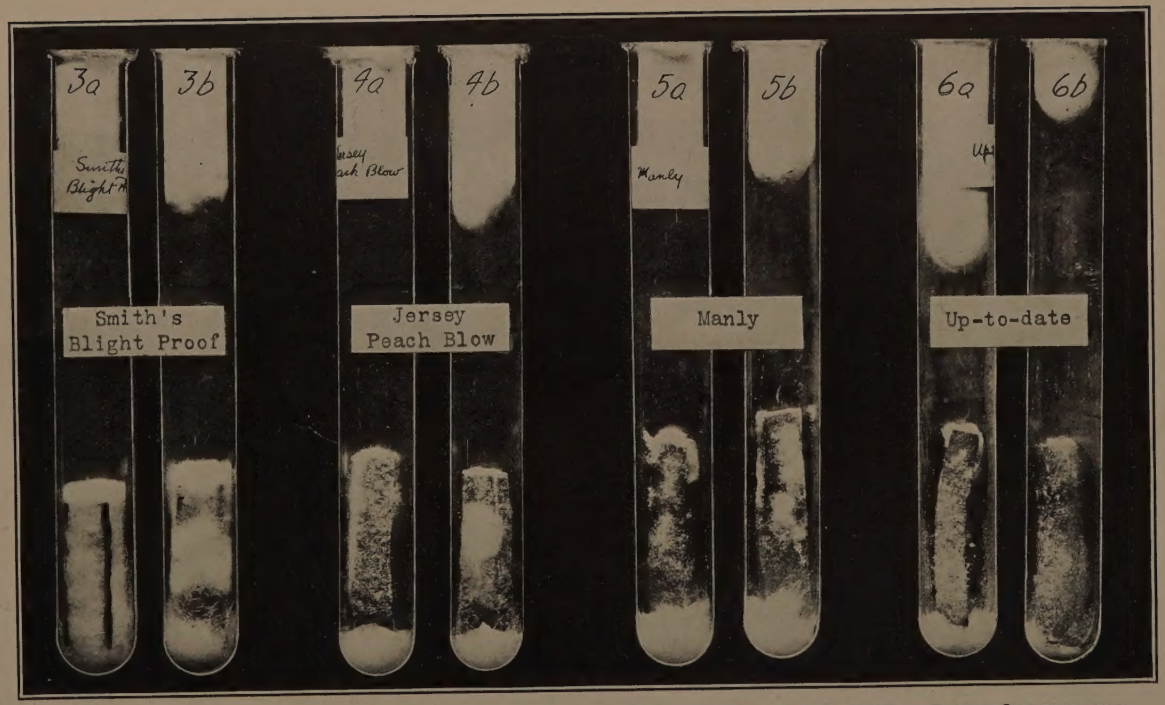


CYTOLOGICAL STUDIES OF PHYTOPHTHORA INFESTANS.

[Figs. 42–47, immature resting-spore structures; fig. 48, mycelium; figs. 49–52, conidia and zoospores (slightly plasmolyzed in fig. 50); fig. 53, haustorium in potato tuber. For further details see "Description of plates" and text.]

PURE CULTURES OF PHYTOPHTHORA INFESTANS, SHOWING 10 DAYS' GROWTH IN TUBES UPON BLOCKS CUT FROM THE INTERIOR OF RAW POTATOES OF VARIETIES WHICH DIFFER IN DISEASE RESISTANCE.

[In the bottom of each tube is a small wad of moist cotton upon which the potato block rests. The German variety Irene (tubes 1*a*–1*d*) shows very little fungous growth—i. e., is highly disease resistant—while the standard American variety Green Mountain (tubes 2*a*–2*d*) shows the vigorous growth characteristic of the highly susceptible varieties. (See also Pl. X.)]



ADDITIONAL PURE CULTURES OF PHYTOPHTHORA INFESTANS, SHOWING 10 DAYS' GROWTH IN TUBES UPON BLOCKS CUT FROM THE INTERIOR OF RAW POTATOES OF VARIETIES WHICH DIFFER IN DISEASE RESISTANCE.

[These cultures show a gradation from the American variety Smith's Blightproof (tubes 3*a*, 3*b*), highly susceptible, through Jersey Peachblow (4*a*, 4*b*) and Manly (5*a*, 5*b*) to the highly disease-resistant English variety Up-to-Date (6*a*, 6*b*). This laboratory method of determining disease resistance promises to be both more reliable and more expeditious than field trials. (For details see "Description of plates" and text, especially Table XI.)]